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Function-regulating pharmacophores in a sulfonamide class of glucocorticoid receptor agonists

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ABSTRACT

A class of α-methyltryptamine sulfonamide glucocorticoid receptor (GR) modulators was optimized for agonist activity. The design of ligands was aided by molecular modeling, and key function-regulating pharmacophoric points were identified that are critical in achieving the desired agonist effect in cell based assays. Compound **27** was profiled *in vitro* and *in vivo* in models of inflammation. Analogs could be rapidly prepared in a parallel approach from aziridine building blocks.

The glucocorticoid receptor (GR) is a member of the nuclear hormone receptor superfamily (NR) that also includes the mineralocorticoid (MR), progesterone (PR), estrogen (ER), and androgen (AR) receptors.[1,2] Endogenous glucocorticoid (GC) agonists, such as cortisol, play important roles in homeostasis. They also participate in the resolution of inflammatory conditions by preventing the transcription of pro-inflammatory genes that lead to the synthesis of cytokines (e.g., IL-1, IL-2, IL-6 and TNF), and adhesion molecules.[3] The anti-inflammatory effects of endogenous GCs prompted the development of synthetic GC agonists such as prednisolone and dexamethasone (Fig. 1); however, the use of GC therapy has been limited due to the side effects associated with chronic dosing.[4] These side effects occur as a result of homeostatic disruption and include alterations in fluid and electrolyte balance, edema, weight gain, hypertension, muscle weakness, diabetes, and/or steroid induced osteoporosis.[5] Furthermore, cross-reactivity of GCs with other NRs, especially MR and PR, may also lead to a number of side effects.

Over the last decade, we and others have reported on a number of non-steroidal GR ligands targeting an improved anti-inflammatory profile with reduced side effects.[6] We have also reported that for a trifluoromethyl carbinol series of GR modulators (compound

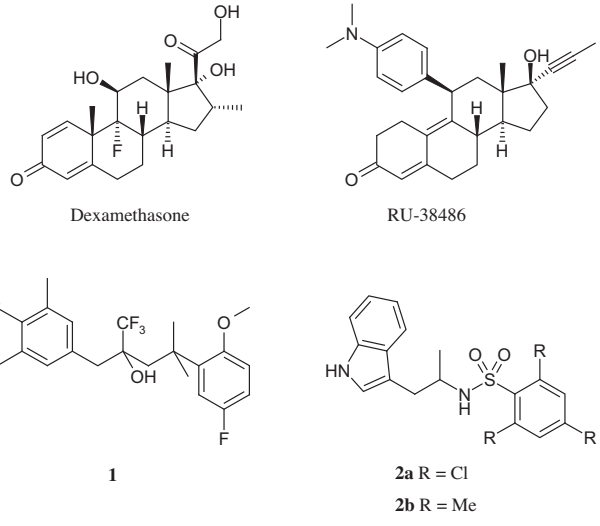

Figure 1. Synthetic glucocorticoid ligands: dexamethansone (agonist), RU-38486 (antagonist), **1** (agonist), **2a** and **2b** (GR modulators).

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1) small structural changes can have a range of effects on potency and efficacy in cell based assays for agonist activity while GR binding potency is maintained.[7] These changes are in contrast to the classical GC antagonist, RU-38486 (Fig. 1), which upon binding to the GR ligand-binding-domain (LBD) causes helix 12 to adopt an open conformation resulting in functional antagonism or 'active antagonism'.[8] Similarly, the sulfonamide series of GC modulators described in this report (e.g., compound **2a**) display a range of effects on potency and efficacy in cell based assays for agonist activity while GR binding potency is often maintained.

Our initial report disclosed the identification of α-methyltryptamine sulfonamide derivatives (e.g., **2a** and **2b**) as potent GR ligands, that were optimized for binding potency and NR selectivity.[9] These compounds failed to display the desired agonist activity in functional assays and were assumed to be GR antagonists. Since our initial publication, a number of groups have reported modifications to the sulfonamide template that have afforded GR agonist activity.[10,11] In this Letter, we describe our SAR findings for this class of GR ligands. Specifically, we report that the placement of function-regulating pharmacophoric points in three distinct regions of this scaffold afford a switch in functional activity providing potent and efficacious GR agonists relative to dexamethasone in a cellular assay.[12,13]

The IC_{50} values reported herein for binding to GR, MR and PR were determined by using a fluorescence polarization competitive binding assay.[14] The GR and MR assays measured the ability of test compounds to compete with tetramethylrhodamine (TAMRA)-labeled dexamethasone. The PR binding assay was run using TAMRA-labeled mifepristone (RU-38486). Anti-inflammatory activity (transrepression) is reported as the inhibition of IL-6 production in human foreskin fibroblasts (HFF) in response to stimulation by the pro-inflammatory cytokine IL-1.[15] It is known that circulating IL-6 levels increase during an inflammatory response and IL-6 production can be inhibited by GCs such as dexamethasone. In the IL-6 functional assay, potency and efficacy are reported, and efficacy is expressed as a percentage of the maximum response observed for dexamethasone.

To guide our SAR and placement of pharmacophoric points we docked compound (***S***)-**2b** into the published GR-LBD binding pocket (Fig. 2).[16] A proposed binding pose of compound (***S***)-**2b** (Fig. 2b) revealed several interesting features. The central NH group of (***S***)-**2b** forms a key H-bond with Asn564 (helix 12) mimicking the interaction seen with the 11β-hydroxyl group of dexamethasone (Fig. 2).

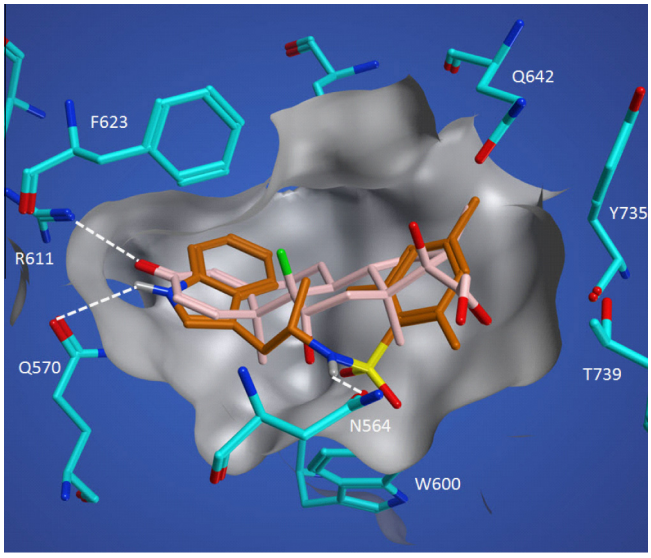

Figure 2. Overlay of docking pose of compound (***S***)-**2b** (orange) with the co-complex x-ray structure of GR-LBD and dexamethasone (rose). Docking was performed using Glide[17] and the picture was generated with MOE.[18] Dotted lines represent hydrogen bonds.

The indole moiety of (***S***)-**2b** overlays with the steroid A-ring of dexamethasone forming π-stacking interactions with Phe623. The tri-substituted phenyl occupies the space of the steroid D-ring. Based on this analysis and our experience optimizing the trifluoromethyl carbinol series (compound **1**) we targeted three strategies for facilitating a functional switch of **2b**. These included: (i) introduction of a polar residue on the phenyl sulfonamide moiety which could engage the Asn564, (ii) replacement of the α-methyl group with a trifluoromethyl moiety to improve the hydrogen bond potential of the sulfonamide N–H, and (iii) placement of a polar residue, useful as a steroid A-ring mimetic, on the indole ring to engage the Arg611 and Gln570 pair. As a proof of concept, compound **3**, containing an aniline moiety designed to engage Asn564, displayed potent GR binding, good potency (IC_{50} = 120 nM) and partial efficacy (59%) in the IL-6 assay. In comparison, compounds **2a** and **2b** are only 12 and eightfold less potent in the GR binding assay, respectively, but were not active in the IL-6 assay (max. concn = 2000 nM). Replacement of the α-methyl group of **3** with a trifluoromethyl group (compound **4**) further improved potency and efficacy in the IL-6 assay relative to compound **3**. Interestingly, the CF_3 modification alone was not sufficient to afford potency or efficacy in the IL-6 assay for compound **5**, the 2,4,6-trichlorophenyl substituted sulfonamide. However, weak efficacy in the IL-6 assay was observed for compound **6**, the 2,4,6-trimethylphenyl substituted sulfonamide. The gem-dimethyl analog **7** also failed to demonstrate efficacy in the IL-6 assay. Clearly, these results highlight the value in a multi-component SAR approach to achieving functional activity. It should be noted that compounds **3**, **4** and **7** demonstrated excellent selectivity relative to dexamethasone in the PR and MR assays. For example, compound **3** was 102-fold more selective for GR over MR in the binding assays (Table 1).

Finally, we focused our attention on the indole moiety which modeling suggests aligns with the same pocket occupied by the A-ring of dexamethasone. Our choice of functional groups was driven by our experience in the trifluoromethyl carbinol series (compound **1**).[7b] In the carbinol series, the cyano group of **1** can serve as a function-regulating pharmacophore due to critical polar interactions with the Arg611/Gln570 pair and a lipophilic component (methyl groups) in this same region can influence the level of agonist activity and efficacy observed. The use of a cyano group as a replacement for the A-ring carbonyl is known from other nuclear hormone receptors, like progesterone[19] and androgen[20] receptor.

To study polar and lipophilic modifications to the indole moiety we chose the 2,4,6-trimethylphenyl sulfonamide analog **6**, and prepared methylindoles **8**–**11** and cyanoindoles **12**–**15** (Table 2). Interestingly, the methylated indole analogs **8**–**11** are all of similar potency in the GR binding assay (GR IC_{50}'s = 10–37 nM). However, a range of GR binding potencies is observed for the cyano analogs **12**–**15**. Thus, the 4-cyanoindole **12** was not potent at the highest concentration tested (GR IC_{50} >10 μM). However, the 7-cyanoindole **15** (GR IC_{50} = 10 nM) is 28 and 32-fold more potent than 6-cyanoindole **14** and 5-cyanoindole **13**, respectively. Consistent with a lipophilic component in this region useful for improving functional activity, the 5-methylindole (compound **9**) afforded partial agonist activity (60% efficacy) in the functional assay. At the same time, consistent with our binding hypothesis (Fig. 2b) that a polar component in the steroid A-ring region is useful for functional activity, the 7-cyanoindole analog **15**, which can engage the Arg611/Gln570 pair, displayed potent binding activity and partial agonism (70% efficacy) in the functional assay. Compound **16**, containing both of these function-regulating pharmacophoric points (the 5-methyl-7-cyanoindole), gave further improvements