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The discovery of potent and selective non-steroidal glucocorticoid receptor modulators, suitable for inhalation

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ABSTRACT

We report the discovery of highly potent and selective non-steroidal glucocorticoid receptor modulators with PK properties suitable for inhalation. A high throughput screen of the AstraZeneca compound collection identified sulfonamide **3** as a potent non-steroidal glucocorticoid receptor ligand. Further optimization of this lead generated indazoles **30** and **48** that were progressed to characterization in in vivo models. X-ray crystallography was used to gain further insight into the binding mode of selected ligands.

Glucocorticoids represent the most potent anti-inflammatory drugs available today and allow successful treatment of several chronic inflammatory and autoimmune diseases. This includes the treatment of respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD), where inhaled glucocorticoids (IGCs) alone or in combination with bronchodilators are the mainstay of clinical therapy. However, there is still a large unmet clinical need in both of these diseases.[1]

Glucocorticoids exert their function through binding to the glucocorticoid receptor (GR). Following ligand binding the cytoplasmic receptor translocates to the nucleus, where it regulates gene transcription by both activating and repressive mechanisms. In analogy with other nuclear hormone receptors, the GR serves as an assembly point for transcription coregulators that can directly modify chromatin structure and/or impact the activity of the gene transcription apparatus.[2] Modulation of receptor activity with non-steroidal ligands, through differential coregulator recruitment can be envisioned as a route to develop GR ligands with improved efficacy and/or safety compared to conventional steroids. This vision is supported by related work in the field of non-steroidal ligands acting on the estrogen and androgen receptors.[3]

During the last decade non-steroidal GR ligands have been identified by large number of organizations.[4] Two examples of compounds reaching clinical studies for topical and oral administration respectively are Mapracorat (**1**) and PF-04171327 (**2**) in Figure 1.[5] Furthermore, the development of novel non-steroidal GR agonists has been facilitated by increased understanding of the structural biology of GR.[6]

We initiated a search for inhaled GR modulators for the treatment of respiratory diseases. A high throughput screen of the AstraZeneca compound collection using a competitive human GR fluorescence polarization binding assay identified the sulfonamide **3** as a potent ligand (Fig. 2). This compound was attractive as it could be synthesized in one step from commercially available starting materials. Further profiling of functional cellular activity using a reporter gene assay system, wherein gene transrepression (TR) activity was determined in Chago K1 cells stably transfected with a construct containing several TRE sites (AP-1 pathway) preceding a Lac Z reporter, revealed the compound to be a functional antagonist. An initial key objective was therefore to introduce functional agonism in this series.

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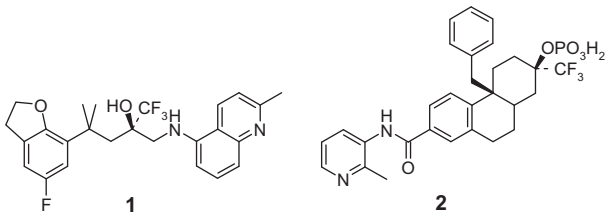


Figure 1. Mapracorat **1** and PF-04171327 **2**.

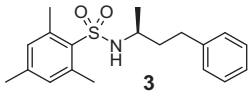
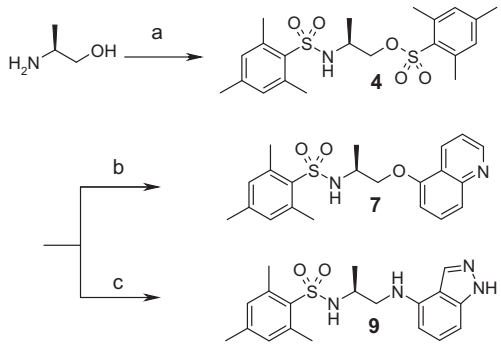


Figure 2. HTS hit **3**.

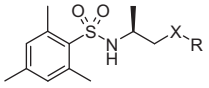
As a first step to improve the synthetic scope, a heteroatom was introduced in the benzylic position in compound **3** to allow parallel chemistry to be used in expansion of the series. A study of the activity of various aryl ethers and anilines was then initiated, using a synthetic route that enabled preparation of ethers (entries **5**–**8**) and amines (entries **9**–**14**) as single enantiomers and exemplified by the synthesis of compounds **7** and **9** in Scheme 1.

Compounds were tested for GR binding and cellular activity in the TR reporter gene assay. For several compounds, affinity to the receptor was retained or improved compared to compound **3** (Table 1). Interestingly, the attachment position to the heterocyclics exhibited a steep SAR. For example, the quinoline (**10**) linked through the 5-position has an affinity to GR of 75 nM whereas affinity is completely abolished for the 6-linked quinoline derivative (**11**). This observation is supported by modeling studies in which the elongated ligand binding pocket in the receptor strictly dictates conditions for the width of compounds. From Table 1 it is also clear that the positioning of aromatic heteroatoms plays an important role for functional activity. While the 5-isoquinoline (**6**) is more potent than the 5-quinoline (**7**) in the binding assay, only the 5-quinoline is an agonist in the cellular TR assay. This can be understood from the perspective that modeling studies place the heterocyclic ring in the same region of the ligand binding pocket as the A and B rings of conventional steroids. For optimal interactions to the receptor, the heteroatom of the heterocyclic ring must emulate the 3-keto oxygen of steroids to pick up key interactions with Arg611, Glu570 and a conserved water molecule.[7] These interactions between helices 3 and 5 of GR have been shown to be critical drivers for agonism.[8]



Scheme 1. Reagents and conditions: (a) 2-mesitylenesulfonyl chloride (2.1 equiv), pyridine, rt, 16 h; (b) 5-hydroxyquinoline (1.7 equiv), DMF, $CsCO_3$, rt, 16 h; (c) 4-aminoindazole, NMP, 130 °C, 2 h.

Table 1
GR binding and activity in TRE reporter gene assay for selected compounds



Compd	X	R	GR[a] IC_{50} (nM)	TR agonistic mode[a]	
				IC_{50} (μM)	Eff.[b] (%)
3	C	Phenyl	55	NA	–
5	O	2,6-Dimethylphenyl	85	NT	–
6	O	5-Isoquinoline	18	NA	–
7	O	5-Quinoline	240	0.96	71
8	O	4-Indole	23	NA	–
9	N	4-Indazole	42	0.76	57
10	N	5-Quinoline	75	1.8	88
11	N	6-Quinoline	10,000	NA	–
12	N	6-Isoquinoline	10,000	NA	–
13	N		18	1.1	59
14	N		160	2.2	56

NT: not tested. NA: not active (<50% inhibition) at 10 μM.
[a] Values are means of at least two experiments.
[b] % Of dexamethasone at 10^{-6} M.

Next, the sulfonamide substituent was explored using a route exemplified for the synthesis of the quinoline (**20**) and isoquinoline (**26**) ethers in Scheme 2.

A range of sulfonamides of different sizes were examined, exemplified by compounds **18**–**27** in Table 2. All substituted phenyl sulfonamides (entries **18**–**25**) showed inferior GR binding compared to the corresponding mesityl sulfonamides. Modeling places these substituents in an area corresponding to the steroid D-ring 17α position and the data confirm the critical role of this pocket for binding affinity. The fact that the potent 1,2-diazoles (entries **26** and **27**) are much larger than the original mesityl also illustrates the tendency for flexibility in this region of the receptor, as previously reported.[9] Since the 1,2-diazoles also exhibited a decrease in lipophilicity (log*D* for matched pairs **6** and **26**, were 4.1 and 3.5, respectively), they were selected for further investigations together with the more potent mesityl congener.

In an effort to emulate the phenyl-pyrazole A-ring substituents in steroid derivatives, such as in cortivazole, a series of 1-aryl-1*H*-indazoles were synthesised.[10] Indazoles **30**–**42** and **47**–**52** (Table 3) were prepared as exemplified by the synthesis of compounds **30** and **47** (Scheme 3).[11] The O, C, S and SO_2 linked 1-aryl-1*H*-indazoles **29**, **43**–**45** and the CF_3 branched indazole ether **46**, were synthesized by different routes.[12]

Many compounds showed excellent potency in the binding and TR assays. The activity of compounds in primary cells were studied in human peripheral blood mononuclear cells (hPBMCs), where the release of tumor necrosis factor-α (TNF-α) in response to lipopolysaccharide (LPS) stimulation was used to test the anti-inflammatory effects. As can be seen from data in Table 3, several of the compounds were confirmed to be potent agonists in this assay.

Functional agonism is driven by a well defined structural state of the receptor, where coactivators can be recruited to the binding surface outlined by helix 3, 4 and 12 (Fig. 3).[7] To better understand the molecular details of the conformations induced by the sulfonamides, we determined the X-ray structure of the GR ligand binding domain (LBD) in complex with compound **30**.[13]

The structure revealed that the compound makes multiple interactions to helix 3, stabilizing it in an agonistic conformation (Fig. 4A). Specifically the nitrogen of the sulfonamide linker makes a direct interaction to the O_δ atom of Asn564. In addition, one of