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The first X-ray crystal structure of the glucocorticoid receptor bound to a non-steroidal agonist

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ABSTRACT

The amino-pyrazole 2,6-dichloro-*N*-ethyl benzamide **1** is a selective GR agonist with dexamethasone-like in vitro potency. Its X-ray crystal structure in the GR LBD (Glucocorticoid ligand-binding domain) is described and compared to other reported structures of steroidal GR agonists in the GR LBD (3E7C).

Steroidal agonists of the Glucocorticoid Receptor (GR) such as prednisolone, dexamethasone or fluticasone esters have potent anti-inflammatory and immunosuppressive properties, and are prescribed medicines used to treat a pleothera of ailments. However, the characteristic side-effects of steroid therapy such as glucose intolerance, muscle wasting, skin thinning and osteoporosis are an undesired outcome of GR activation. In addition, cross-reactivity of steroids with the progesterone receptor (PR) androgen receptor (AR) and mineralocorticoid receptor (MR) can provoke off-target pharmacology.[1] Thus in recent years considerable effort has been directed towards finding non-steroidal ligands that maintain the desired anti-inflammatory properties while limiting negative side-effects.[2–4]

It has been surmised that non-steroidal ligands could make subtly different interactions with the GR LBD compared to the steroids, These modified interactions may result in varied and desirable pharmacologies. An understanding of how such molecules bind in the GR LBD would therefore be valuable for future rational drug design efforts. We and several other groups have recently found that an aryl indazole or pyrazole group is a valid substitution for the steroidal A-ring, reminiscent of that moiety present in cortivazol.[5–9] We have previously reported the X-ray crystal structures of dexamethasone[10] and fluticasone furoate (FF)[11] in the GR LBD and Xu has recently published the structure of GR LBD bound to deacylcortivazol (DAC)[12] (Fig. 1). We now report the first structure of the GR LBD with a bound non-steroidal ligand.

In the accompanying letter,[13] we report a series of aryl amino-pyrazole benzamides which are GR selective agonists. One of the most potent compounds was the *N*-ethyl 2,6-dichlorobenzamide **1** (Fig. 1). A pIC_{50} of 8.5 was observed in the GR-binding assay and the molecule was a full agonist in the anti-inflammatory NFκB assay in A549 cells and in the MMTV transactivation assay (pEC_{50} 8.7). Nuclear receptor selectivity was evidenced by its poor binding to AR, PR, and MR and lack of activity in cellular assays ($pEC_{50} < 5$).

As we discussed in the accompanying letter,[13] modeling of **1** in the FF–GR structure (Fig. 2) placed the *N*-ethyl group in the same position in the protein as the putative position of the trigger group in our tetrahydronaphthalene (THN) series. The aryl amide group was predicted to occupy the hydrophobic pocket populated by the furoate group in FF. The central OH was predicted to form the crucial H-bond to N564 as in the THN series and mimics the steroidal 11β-OH. Q570 was predicted to make an H-bond with pyrazole N5 and R611 to be displaced from its H-bonding location with dexamethasone.

The GR LBD protein was the same construct and was purified and complexed by the same protocol as described in the GR–FF letter.[11] The crystal structure has been deposited in the RCSB as PDB code: 3E7C. The initial crystals of GR LBD complexed with **1** were obtained from the following crystallization conditions: 0.1 M MES 6.5 and 1.6 M magnesium sulfate. The crystals were optimized after a second preparation of protein to 2.0 M magnesium sulfate

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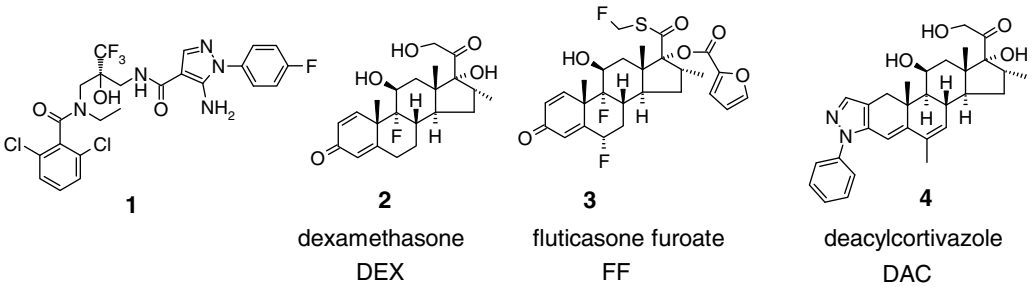

Figure. 1. GR agonist ligands in crystal structures bound to GR LBD that are discussed in this letter.

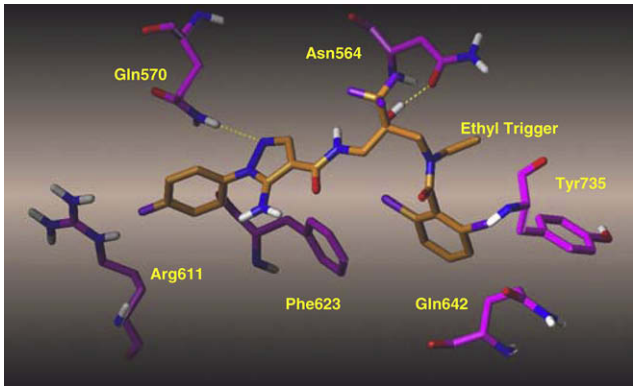

Figure. 2. The model of **1** based on the FF crystal structure, as described in the companion letter.

with 0.1 M MES 6.5 and the additive β-hexylglucoside. The crystals were frozen by a quick dunk method with well conditions plus 30% glycerol. The data was reduced and scaled using *HKL2000*.[14] Molecular Replacement was achieved with *Phaser*,[15] and the structure was built using *COOT*[16] and refined using *Phenix*.[17] The structure figures were made using *Pymol*.[18]

The crystal structure of the LBD of GR with bound ligand **1** was solved to a resolution 2.15 Å and is shown in Figures 3 and 4. The crystallographic data and refinement statistics are shown in the Table 1. This ligand extends the binding pocket into the 17α pocket beyond the D ring of dexamethasone (Fig. 5) with the di-chlorophenyl group and into a pocket beyond the A ring of a standard steroid with the fluoro-phenyl group. The conserved steroidal A ring charged clamp region of steroid receptors is disrupted by

Table 1
X-ray crystallographic data for **1** bound into GR ligand binding domain

Crystallographic data		Refinement statistics	
Space group	*P61*	Resolution range	20–2.15
a, *b*, *c* (Å)	126.5, 126.5, 78.4	% *R*free	7
α, β, γ (°)	90, 90, 120	*R*cryst (*R*free) (%)	20.9 (26.65)
Molecules in ASU	2	Protein atoms	3936
Resolution range (Å)	50–2.15	Ligand atoms	72
Completeness (final shell)	96.4 (91.7)	Solvent molecules	94
I/σ (final shell)	41.3 (1.8)	Rmsd bonds (Å)	0.005
*R*merge (%) (final shell)	4.7 (47.8)	Rmsd angles (°)	0.701

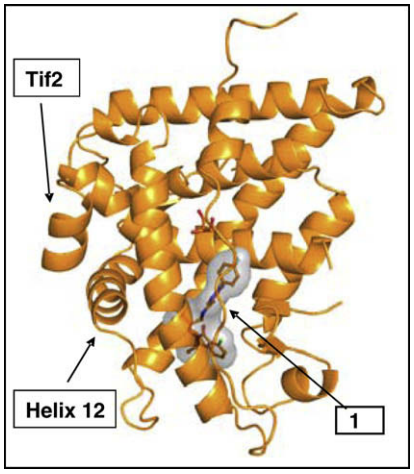

Figure. 4. Orientation of **1** in GR LBD as seen in crystal structure.

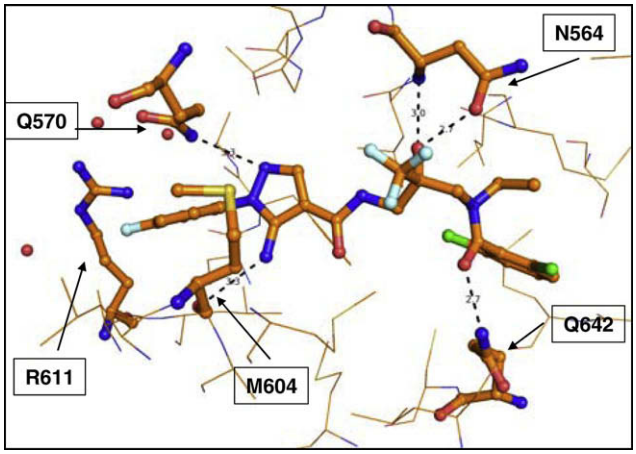

Figure. 3. Binding pocket of **1**/GR LBD as seen in X-ray crystal structure.

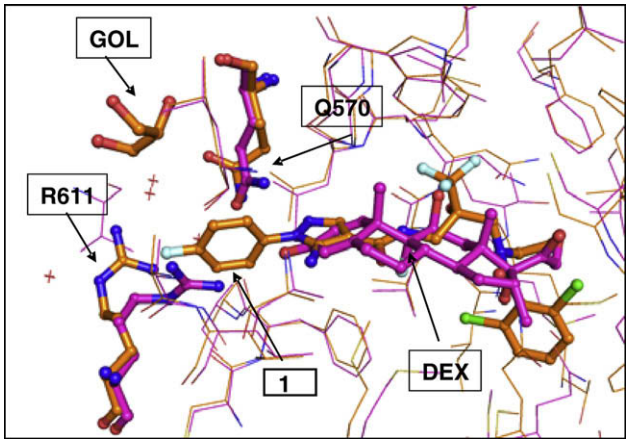

Figure. 5. Overlay of crystal structures of **1** (gold) and dexamethasone (magenta) bound to GR LBD.

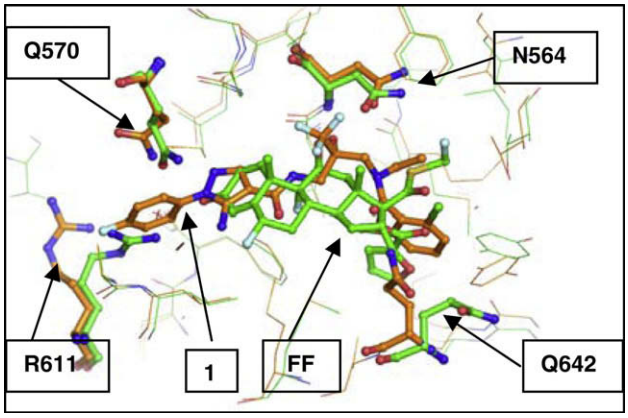

Figure. 6. Overlay of the crystal structures of **1**(gold) and FF (green) bound to GR LBD.

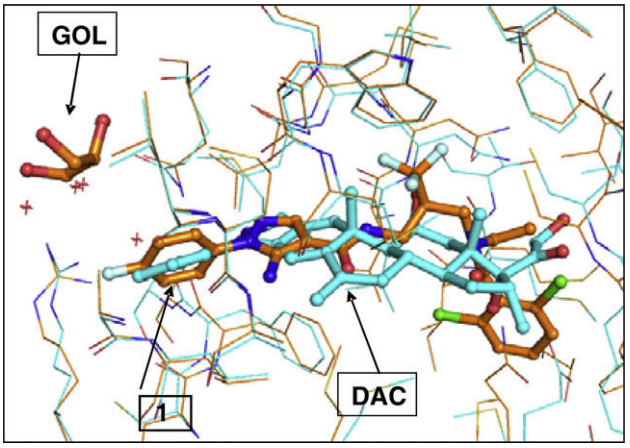

Figure. 7. Overlay of the crystal structures of **1**(gold) and DAC (blue) bound to GR LBD.

the movement of Q570 and R611 from the position seen in dexamethasone. Instead Q570 and R611 are involved in a water-mediated hydrogen bonding network and Q570 H-bonds to the pyrazole nitrogen of the ligand as we had predicted. The amide carbonyl of the aminopyazole forms an internal H-bond with the NH_2 also as predicted, and this helps to flatten the ligand to adopt a geometry similar to that seen with a steroid. The Q642 H-bond to the ligand is an interaction reminiscent of the H-bond to the 21-OH in dexamethasone, but this was not predicted in the model. Otherwise, the model was fairly accurate.

A comparison of the GR binding pockets observed in the dexamethasone[4] (pdb id: 1m2z) and fluticasone furoate (FF)[11] (pdb id: 3cld) with the deacylcortivazol (DAC)[12] (pdb id: 3bqd) and **1** crystal structures reveal that GR readily changes conformation to accommodate large moieties on the ligands.

The 17α pocket of GR disclosed in our recent structure of FF[11] accommodates the furoate ester group. Compound **1** extends into this 17α pocket deeper than dexamethasone, but not to the extent of FF, as seen in Fig. 6. **1** is seen overlaid with the recent GR LBD-DAC structure in Figure 7. DAC contains a large arylpyrazole A-ring extension but is otherwise similar to dexamethasone. The A-ring arylpyrazole extension in DAC overlays well with the arylpyrazole in **1**, as do the hydroxyls which H-bond to N564. There is no 17α extension in DAC.

Dexamethasone occupies the smallest binding pocket of the known GR agonist ligands (Fig. 5). In contrast, the DAC and FF crystal structures reveal how these ligands can extend the binding pocket in 2 directions beyond that observed with dexamethasone. The non-steroidal ligand **1** utilizes both opportunities for pocket extension. Despite the 2 extensions, the position of helix 12 (a helix associated with functionality in nuclear receptors) is unaffected as is the cofactor Tif2 that binds to it. Furthermore, there is a glycerol molecule (GOL) seen in both copies of the molecule that is 4 Å from the fluoro-phenyl ring of **1** (Fig. 5), suggesting that a ligand can be extended with hydrophilic groups even further into pocket occupied by the fluoro-phenyl group. This provides scope for the design of further non-steroidal GR ligands.

With the insights gained as the number of crystal structures in GR increases, modeling for future medicinal chemistry will become more accurate. The ability to reach these extended pockets with non-steroidal compounds may be important to achieve selectivity over the other steroid nuclear receptors and dissociated pharmacology.

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