Letters

X-ray Crystal Structure of the Novel Enhanced-Affinity Glucocorticoid Agonist Fluticasone Furoate in the Glucocorticoid Receptor−Ligand Binding Domain[⊥]

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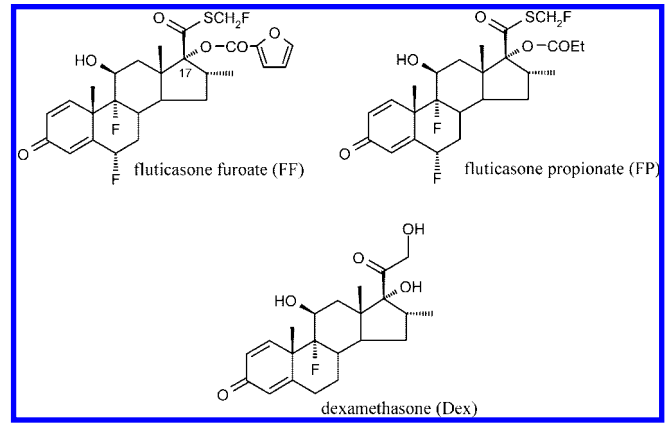
Abstract: An X-ray crystal structure is reported for the novel enhanced-affinity glucocorticoid agonist fluticasone furoate (FF) in the ligand binding domain of the glucocorticoid receptor. Comparison of this structure with those of dexamethasone and fluticasone propionate shows the 17α furoate ester to occupy more fully the lipophilic 17α pocket on the receptor, which may account for the enhanced glucocorticoid receptor binding of FF.

Fluticasone furoate (FF,[a] GW685698X) is a novel topical glucocorticoid recently introduced for the intranasal treatment of seasonal and perennial allergic rhinitis.[1] FF is a potent lipophilic glucocorticoid agonist displaying once-daily efficacy on both nasal and ocular symptoms of allergic rhinitis.[2] FF combines the fluticasone template, which confers efficient systemic inactivation through the 17β-fluoromethylthioester moiety, with a metabolically stable 17α furoate ester. The efficient hepatic inactivation of FF results in negligible systemic exposure (<1%) after intranasal dosing, and levels of free drug are further minimized by the very high plasma protein binding (>99%) of FF.[3,4]

The furoate ester of FF replaces the simpler propionate ester of the earlier fluticasone propionate (FP). This 17α elaboration has been shown to confer enhanced respiratory tissue retention and receptor binding for FF compared to FP, properties believed to contribute to its attractive clinical profile.[4,5] Thus, FF displays a rapid association with and slow dissociation from the human glucocorticoid receptor (GR) resulting in the highest reported receptor binding affinity (2989 ± 135 with reference to dexamethasone 100, fluticasone propionate 1775 ± 130).[5]

The structure of the ligand binding domain of the glucocorticoid receptor (GR) was first determined through X-ray crystallography in 2002 as a complex with dexamethasone (Dex).[6] Prior to that time we had utilized the structure for the closely homologous progesterone receptor (PR), with progesterone bound, to provide insights into interactions between steroidal ligands and GR.[7] It was clear from the PR structure that a small lipophilic pocket existed close to the 17α steroidal position and that by analogy a similar, but larger, pocket must exist in GR to accommodate the 17α propionate group of fluticasone propionate (FP). Early studies with dermatological glucocorticoids established that topical activity could be enhanced by the introduction of lipophilic 17α ester functionality such as simple alkyl and benzoate esters.[8] A small number of heteroaryl esters were investigated in the 1980s[9] leading to the identification of mometasone furoate[10] and, more recently, relatively simple 17α carbonate esters have also been explored leading to the discovery of loteprednol etabonate.[11] However, when we started this work, no detailed investigation of the effects of 17α esterification had been described[12] and we therefore undertook a program of work to extensively investigate 17α elaboration on the fluticasone template.[13] This work led to the identification of the 2-furoate ester of fluticasone (fluticasone furoate, FF) displaying very interesting properties including the highest glucocorticoid receptor affinity so far reported.[4,5]



Subsequent to this original modeling work, crystal structures for GR complexed with Dex, FP, and FF were obtained allowing comparison of the ligand−receptor interactions and a qualitative and semiquantitative rationalization of the differences seen in binding affinity. The crystal structures for complexes with Dex[6,14] and FP[15] have been published elsewhere, but here the crystal structure for the FF complex is published for the first time. Crystal structures for GR complexes are rare. However, the structure for GR complexed with the antagonist RU486 has been determined[14] and very recently the structure was reported for GR complexed with the agonist deacylcortivazol.[16]

Fluticasone furoate was readily prepared[13a] from the known hydroxy thioacid **1** using standard methodology.[17] Reaction of **1** with excess 2-furoyl chloride (2.6 equiv) followed by treatment of the intermediate bis-acylated 17β mixed anhydride with diethylamine afforded the thioacid 17α furoate **2**. Alkylation of **2** with bromofluoromethane then gave fluticasone furoate as a white crystalline solid (Scheme 1).

The human glucocorticoid receptor (residues 521−777 F602Y, C638G) was expressed as a 6xHisGST fusion protein in *E. coli* supplemented with FF. Purification of the GR ligand binding

[⊥] The structure of the fluticasone furoate glucocorticoid receptor–ligand binding domain complex has been deposited in the Brookhaven Protein Data Bank (PDB code 3CLD).

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[a] Abbreviations: FF, fluticasone furoate; FP, fluticasone propionate; Dex, dexamethasone; GR, glucocorticoid receptor; LBD, ligand binding domain; TIF, transcriptional intermediary factor.

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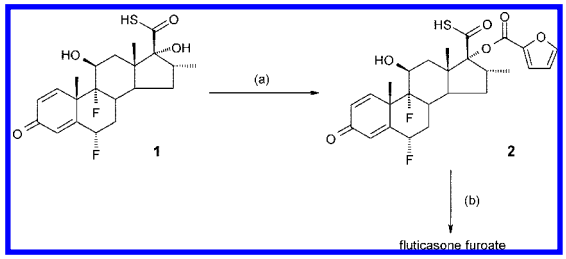
Scheme 1. Synthesis of Fluticasone Furoate[a]



[a] Reagents and conditions: (a) 2-furoyl chloride, triethylamine, CH_2Cl_2, <5 °C, then diethylamine, acetone, room temp, 82%; (b) $BrCH_2F$, $NaHCO_3$, *N*,*N*-dimethylformamide, −20 °C, 88%.

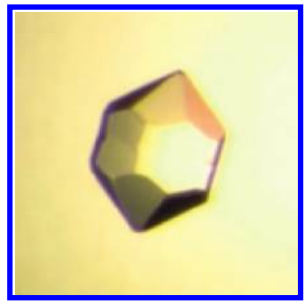


Figure 1. Crystal obtained for GR/FF/TIF2.

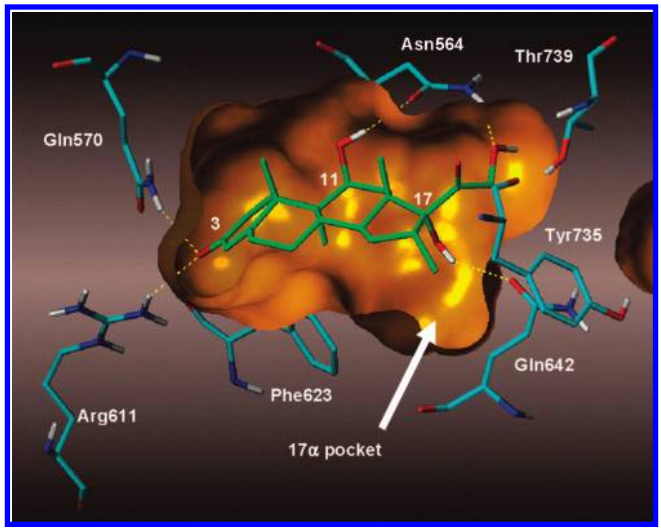


Figure 2. Interactions observed in the Dex GR LBD/TIF2 crystal structure.

domain (LBD) was accomplished by affinity chromatography and thrombin cleavage followed by ion exchange chromatography. Crystals of the GR protein (Figure 1) complexed with a 12-residue TIF2 coactivator peptide were obtained by the hanging drop vapor diffusion method at 22 °C using 100 mM BisTrisPropane, pH 7.0, and 2.2 M sodium chloride as the precipitant. Crystals were flash frozen in paraffin oil prior to data collection and belong to the space group $P6_1$ with 2 mol/asu. The data were collected at beamline 17ID at the Advance Photon Source (APS) at Argonne National Laboratories, and the structure was solved by molecular replacement using the coordinates of GR complexed with dexamethasone as a starting model. The structure was refined with CNX[18] and Refmac[19] to an R_{factor} of 21% at 2.85 Å resolution.[20]

The overall structure of the GR LBD has been previously described.[6,14,21,22] In all three structures, GR/FF/TIF2, GR/FP/TIF2, and GR/Dex/TIF2, the GR LBD binds one steroid molecule and one coactivator peptide. The GR/FF structure was

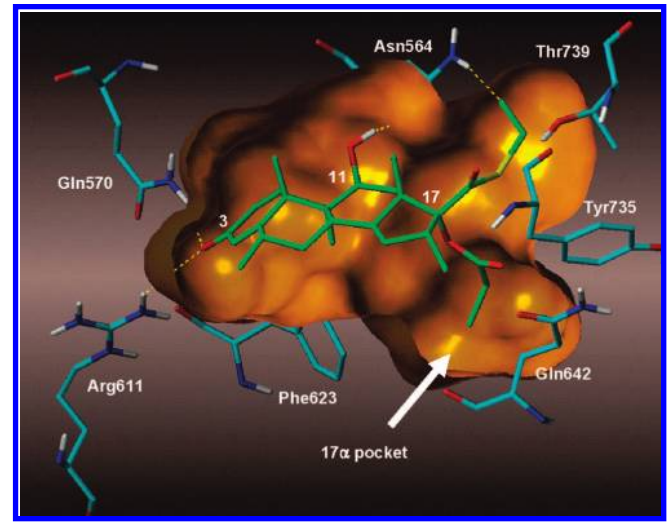


Figure 3. Interactions observed in the FP GR LBD/TIF2 crystal structure.

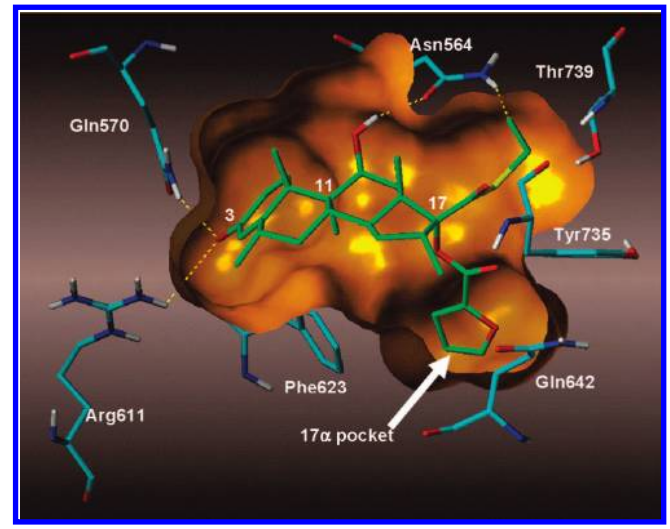


Figure 4. Interactions observed in the FF GR LBD/TIF2 crystal structure.

solved using a mutant variant of the GR LBD different from that used for the Dex and FP complexes, F602Y and C638G, which were introduced to improve expression and purification. These mutations are distant from the ligand binding cavity and do not perturb the overall protein structure.

There are no large conformational changes of helices or loops between the FF, FP, and Dex structures, consistent with the fact that all three agonist ligands bind with high affinity. However, in both the FF and FP structures, the 17α pocket is expanded relative to the pocket observed in the Dex structure because of small movements in helices 3, 6, 7, and 10 and the loop preceding the AF2 helix as well as changes in the conformation of the side chains of Met560, Gln642, and Tyr735. The overall protein conformation and size of the 17α pocket were essentially identical in the FF and FP structures.

Interactions between the protein and ligands (Dex, FP, and FF) are shown in Figures 2–4. It can be seen that for Dex, FP, and FF the 3-keto group forms hydrogen bonds to Gln570 and Arg611 while the 11β hydroxyl forms hydrogen bonds to Asn564. Asn564 also forms a hydrogen bond to the Dex 21-hydroxyl group, and a similar interaction is seen with the 17β-fluoromethylthio fluorine of FP and FF, which can be considered to make a favorable electrostatic interaction with Asn564.

The 17α hydroxyl group of Dex is seen to hydrogen-bond to Gln642, an interaction that is not possible with FP and FF, which replace this hydroxyl with a 17α ester function. However, the absence of the hydrogen bond is more than compensated for by the introduction of favorable van der Waals (VDW) interactions within the 17α pocket which FF appears to fill fully but which FP fills only partially. Indeed, for the FP complex only weak density is observed for this substituent and two different conformations were observed in the two molecules in the asymmetric unit indicating considerable freedom of movement[15] (only one molecule is shown in Figure 3). For FF the key interacting residues in the 17α pocket were seen to be Met560, Leu563, Met639, Gln642, Met643, Met646, Tyr735, Cys736, Thr739, and Ile747.

The relative differences in binding affinity between Dex, FP, and FF are 1:18:29.[5] There are multiple differences between Dex and FP/FF, most notably the loss of two clear hydrogen bonds seen for Dex which are replaced by a favorable electrostatic interaction between the fluorine atom of the fluoromethylthio group and by favorable VDW/hydrophobic interactions in the 17α pocket. H-bonding can clearly be critical for specificity at a target, but the effect of the loss of a hydrogen bond on binding affinity can be highly variable. It is very much dependent on the nature and the quality of the hydrogen bonding interactions that are being replaced; i.e., the ligand will have opportunities to form excellent H-bonds with solvent and it is likely that the protein will likewise have solvent interactions that are displaced on ligand binding. In this case it would appear that the loss of hydrogen bonds can be more than compensated for by increased VDW/hydrophobic interactions through an increase in buried surface area. FP and FF are an interesting comparison because they differ only in the 17α ester moiety. The increase in binding affinity for FF over FP is just over 60%. FF can be seen to fill the lipophilic 17α pocket more effectively than FP (see Figures 3–5). The difference in the 17α pocket lipophilic contact surface area, between FP and FF, was estimated[23] at 14 Å^2. This increase in buried hydrophobic surface would certainly increase the binding affinity. Quantifying such an effect is not straightforward. However, the free energy gain may be calculated using the figures for lipophilic contact proposed by Bohm:[24] −0.17 kJ mol^{-1} Å^2. Applying this figure suggests a FP to FF free energy gain of −2.4 kJ mol^{-1}, which would be expected to increase affinity by 2.5-fold, a result of similar order to that observed.

In summary, the crystallographically determined structure of the GR/FF complex is published here for the first time. Fluticasone furoate is the highest affinity ligand so far identified for GR, and structural comparisons at the molecular level have highlighted the more complete filling of the lipophilic 17α pocket by the furoate moiety as the likely source of this enhanced receptor affinity. The structure of GR/FF has been deposited in the Brookhaven Protein Databank (PDB code 3CLD) and should be of great utility to researchers in the field.

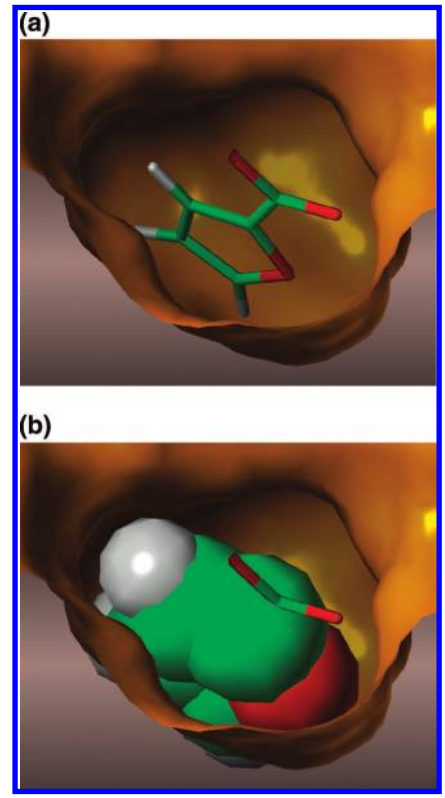


Figure 5. Excellent complementarity between the furoate group and the 17α pocket: (a) furoate in 17α pocket; (b) CPK representation.

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(20) Crystals of GR complexed with FF belong to the space group $P6_1$ with a unit cell of $a = b = 127.34$Åand $c = 77.77$Å. Data were collected from a single crystal and processed with HKL2000. The data between 50 and 2.85Å(15 786 unique reflections) was 96% complete using a 2σcutoff and had an R_{sym} of 7.4%. The structure was refined to an R_{factor}/R_{free} of 21.4/28.5%.

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