Journal of Molecular Graphics and Modelling 27 (2008) 512–521

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

# Induced-fit docking of mometasone furoate and further evidence for glucocorticoid receptor 17α pocket flexibility

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ARTICLE INFO

*Article history:*
Received 4 April 2008
Received in revised form 22 August 2008
Accepted 2 September 2008
Available online 10 September 2008

*Keywords:*
Induced-fit docking
Ligand–protein interaction
Protein flexibility
Glucocorticoids
Glucocorticoid receptor
Mometasone furoate

ABSTRACT

An induced-fit docking method was used to characterize the interactions of the glucocorticoid receptor binding-site with mometasone furoate, a glucocorticoid with a lipophilic ester at the C17α position. Two validation studies demonstrated that the protocol can reproduce crystal structures of nuclear receptors, and is appropriate for modeling ligand binding to the glucocorticoid receptor. Key hydrogen bonding interactions between mometasone furoate and the glucocorticoid receptor, as well as favorable hydrophobic interactions between the furoate group and the 17α pocket, contribute to high affinity and specificity of this ligand for the receptor. Using the glucocorticoid des-ciclesonide, which has an even larger moiety at the 16,17α position, induced-fit docking demonstrates the ability of the 17α pocket of the receptor to expand even further to accommodate the ligand.



## 1. Introduction

Since Hench's pioneering research in the 1950s using hydrocortisone for the treatment of rheumatoid arthritis, glucocorticoid (GC) compounds have become the foundation of management of many inflammatory diseases, including those of the lung and upper respiratory tract such as asthma and allergic rhinitis [1–5]. Older oral GCs such as dexamethasone (Dex) and prednisolone are effective at suppressing inflammation, but produce negative side effects such as hyperglycemia and osteoporosis, particularly with long-term administration [6]. The more recent development of GCs administered by inhalation or intranasal application marked a major advance in the management of upper respiratory inflammatory diseases by providing local drug delivery with minimal systemic absorption. Pharmaceutical research has continued to search for high-affinity GC compounds with minimal toxicity, and the newest GCs for inhalation/intranasal administration (*i.e.*, mometasone furoate [MF], fluticasone furoate [FF], fluticasone propionate [FP] and ciclesonide) have greatly improved benefit-risk ratio.

Glucocorticoid pharmacologic activity is mediated through interaction with the glucocorticoid receptor (GR), a member of the nuclear-receptor family of ligand-activated transcription factors that has been shown to suppress the inflammatory response in the context of asthma [7]. Similar to other members of this family, GR is characterized by three major domains: an N-terminal activation function-1 domain (AF-1), a central DNA-binding domain, and a C-terminal ligand-binding domain (LBD) [8]. An accurate understanding of the structure of the LBD could have tremendous ramifications for glucocorticoid research and pharmaceutical development, but unfortunately this domain is difficult to express in recombinant form and is not easily purified or crystallized [8,9]. To date, three human GR-LBD structures have been documented in the Protein Data Bank (PDB) [8–10]. These structures reveal that GR has a unique side pocket bounded by helices 3, 6, and 7. This pocket can accommodate large substituents at position C17α of GCs that are characteristic of clinically effective compounds. Experimental evidence shows that the GR binding site is extremely flexible and adaptive in its interactions with GCs.

Most modeling of ligand–receptor interactions has been done via docking methods that use rigid receptor structures obtained either from crystallography or homology modeling. Ligands that

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*Abbreviations:* des-CIC, desisobutyryl-ciclesonide; Dex, dexamethasone; FF, fluticasone furoate; FP, fluticasone propionate; GC, glucocorticoid; GR, glucocorticoid receptor; IFD, induced-fit docking; LBD, ligand-binding domain; MF, mometasone furoate; PDB, Protein Data Bank; PR, progesterone receptor; RBA, relative binding affinity; RMSD, root-mean-square deviation; vdW, van der Waals.

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doi:10.1016/j.jmgm.2008.09.002

require conformational changes in the receptor for binding limit the usefulness of these traditional methods. In fact, receptor flexibility is one of the greatest challenges for structure-based drug design [11,12]. The binding-site conformational changes induced by different ligands can range from modest to striking, depending on their interactions with the receptor. In a recent study, Boström *et al.* showed that the binding sites of pairs of proteins complexed with structurally similar ligands differed in 83% of cases [13]. Side-chain movements were observed in half of the pairs, whereas backbone movements rarely occurred. By adopting different rotamers, protein side-chains can cause changes in the shape, size, and electrostatic character of the receptor binding-pocket. Therefore, the use of a single rigid protein structure is in many cases too primitive for accurately docking ligands into receptors [14], and rigid-receptor docking has failed to produce reasonable models when the protein must be "induced" into the correct binding conformation for a given ligand.

Induced-fit docking (IFD) can be used to model and characterize binding-site geometries while taking into account both ligand and receptor flexibility [15–19]. Schrödinger's IFD protocol combines the use of a rigid-receptor docking program (Glide) [17] with a protein structure prediction and refinement module (Prime) [20] to allow accurate prediction of ligand-binding modes and concomitant structural changes in the receptor. Glide was designed to explore the positional, orientational, and conformational space of the ligand within the protein binding-site, while Prime took care of side-chain conformational changes as well as limited backbone changes in protein loop regions. IFD has the potential to produce a structure that more accurately reflects binding interactions by mutually accommodating the receptor and ligand to each other. Furthermore, IFD was shown to be able to generate reasonable binding structures for ligands known to be active but unable to be docked in an existing structure of the receptor using the rigid approach [16].

Certain features of corticosteroid structure–activity relationships appear to be common to all glucocorticoids [21]. For instance, carbonyl groups at C3 and C20, a β-hydroxyl group at C11, and a $\Delta^{4,5}$ double bond are essential for good GR binding [22]. A double bond at C1 generally increases selectivity for GR versus the mineralocorticoid receptor and improves anti-inflammatory activity, as does the combination of halogenation (either chlorine or fluorine) at C6 or C9 and an α- or β-methyl group at C16 [23]. Halogenation at either C6 or C9 increases receptor-binding affinity, but halogen substitutions at both positions do not give further increases in potency [22]. Many glucocorticoids have incorporated substituents at the 17α position to increase binding affinity and lipophilicity. Mometasone furoate (9,21-dichloro-11[β],17-dihydroxy-16[α]-methylpregna-1,4-diene-3,20-dione 17-[2-furoate]), a corticosteroid formulated for inhalation and intranasal use, was the first marketed corticosteroid to incorporate the lipophilic furoate ester at the 17α position (Fig. 1). MF has a high affinity for GR, with reported relative receptor affinity (RRA) values ranging from 1200 to 2900 [23–26]. While the furoate ester has contributed to these characteristics, it has not been demonstrated how the structural features of MF contribute to its high binding affinity, since no experimental or modeled structure of this molecule in complex with GR has been reported to date.

Our primary goal in this study was to apply IFD methodology to gain a detailed understanding of the nature of the MF–GR interactions. This corticosteroid-GR complex is of particular interest because the contacts between the highly desirable furoate moiety of MF and the GR 17α pocket have not yet been fully elucidated. In order to gain further perspective into the conformational flexibility of this pocket, we conducted a similar IFD analysis

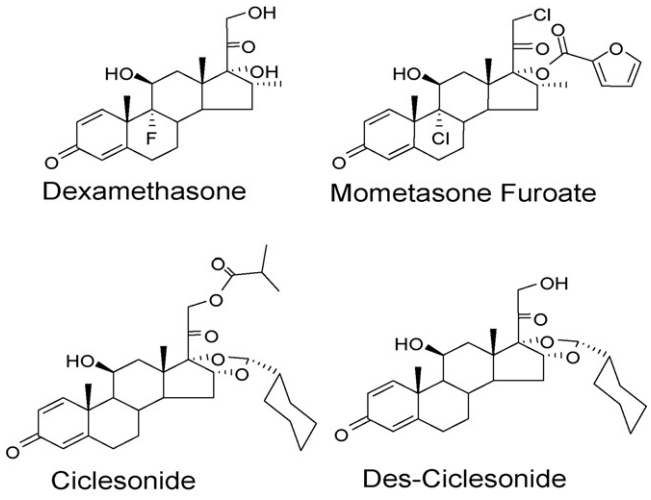


**Fig. 1.** Molecular structures of the compounds studied.

with the glucocorticoid desisobutyryl-ciclesonide (des-CIC), which has an even larger moiety at the 16,17α position (Fig. 1). des-CIC is the active metabolite of the pro-drug ciclesonide ([R]-11[β],16[α],17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with cyclohexanecarboxaldehyde 21-isobutyrate), another corticosteroid formulated for inhalation/intranasal administration. Ciclesonide is converted to des-CIC by esterases at the site of action (*e.g.*, lung/nasal tissue), and the active metabolite has an approximately 100-fold greater RBA for GR than its pro-drug [27,28].

## 2. Methods

### 2.1. Protein structures

The protein structures used in this study were retrieved from the PDB [29] and included the following: GR-LBD in complex with Dex (PDB IDs: 1p93 and 1m2z), GR-LBD in complex with deacylcortivazol (PDB ID: 3bqd), progesterone receptor (PR)-LBD in complex with progesterone (PDB ID: 1a28) [30], and PR-LBD in complex with MF (PDB ID: 1sr7) [31]. All chains in these structures were extracted and aligned using LSQMAN [32] with 1p93 chain A as the template (The brute force option was used to align the structures). Additionally, a GR-LBD structure in complex with FP was documented in a recent patent [33].

For structures with multiple chains, only chain A was retained and prepared for docking studies. Protein preparations were carried out with Maestro [34] and involved the following steps: assign bond orders and add hydrogen atoms to the ligand molecule; add hydrogen atoms to protein heavy atoms and charge the Asp, Glu, Arg and Lys residues; optimize the orientation of hydroxyl groups on Ser, Thr and Tyr residues; optimize the side chains of Gln and Asn residues; and determine the state of His residues. The ligand and water molecules in each structure were retained throughout the protein preparation process. Water molecules and cofactors were removed before docking studies. The binding pocket volume was calculated with VOIDOO using default parameters [35].

### 2.2. Compound preparation

The two-dimensional structures of the compounds used in this study are shown in Fig. 1. Corresponding three-dimensional structures were generated using the Concord program [36]. These

structures were then subjected to energy minimization using MacroModel [37] with Merck Molecular Force Field (MMFFs) and a constant dielectric of 1.0. Energy minimization was terminated when the gradient dropped below 0.05, and the minimized structures were used as the starting points for docking.

### 2.3. Rigid docking

A rigid docking of MF to 1p93 was carried out using Glide XP methodology set at default parameters, unless otherwise specified. Chain A of 1p93, prepared as described above, was used to generate the energy grids for docking. Water molecules and the TIF2 cofactor molecule in the crystal structure were removed, but no energy minimization of the protein structure was conducted prior to grid calculation. The receptor binding-site was represented by the energy grids using a cubic box centered on the centroid of the crystal ligand. Dimensions for the bounding box (within which the centroid of a docked pose is confined) were set to 12 Å × 12 Å × 12 Å. No geometric or hydrogen-bonding constraints were imposed on any of the protein binding-site atoms. A maximum of 10 poses were saved.

### 2.4. Induced-fit docking

The IFD protocol used in this study was carried out in three consecutive steps [16]. First, the ligand was docked into a rigid-receptor model with scaled-down van der Waals (vdW) radii. A vdW scaling of 0.5 was used for both the protein and ligand non-polar atoms. A constrained energy minimization was carried out on the protein structure, keeping it close to the original crystal structure while removing bad steric contacts. Energy minimization was carried out using the OPLS-2001 force field with implicit solvation model until default criteria were met. The centroid and size of the crystal ligand were used to define the location of the binding site and the dimension of the energy grids for initial docking. The Glide SP mode was used for the initial docking, and 20 ligand poses were retained for protein structural refinements.

In the second step, Prime was used to generate the induced-fit protein–ligand complexes. Each of the 20 structures from the previous step was subjected to side-chain and backbone refinements. All residues with at least one atom located within 4.0 Å of each corresponding ligand pose were included in the Prime refinement. All steroidal ligands used in this study had a C3-carbonyl group in their A-rings that forms identical interactions as in the crystal structures. Since we do not expect any significant conformational changes in this region, two of the binding-site residues that make hydrogen bonds with this carbonyl group (Gln570 and Arg611 in GR; Gln725 and Arg766 in PR) were fixed during side-chain refinement. In both the GR and PR crystal structures, these two residues also make extensive hydrogen bonds with water molecules in a nearby pocket. Since water molecules were removed in the IFD studies, fixing the conformation of these residues would also reduce any artifacts caused by the water cavity. The refined complexes were ranked by Prime energy, and the receptor structures within 30 kcal/mol of the minimum energy structure were passed through for a final round of Glide docking and scoring.

In the final step, each ligand was redocked into every refined low-energy receptor structure produced in the second step using Glide XP at default settings. An IFD score that accounts for both the protein–ligand interaction energy and the total energy of the system was calculated and used to rank the IFD poses. The best-ranked IFD structures were used for comparison with the available crystal structures or as a model for new GCs. The root-mean-square deviations (RMSDs) between the docked ligands and the corresponding crystal structures were calculated after aligning protein Cα atoms, and only heavy atoms were used for the calculations.

### 2.5. Hydrophobic interaction map

A hydrophobic interaction map of the binding site of the GR-LBD, which allows the visualization of the locations of the hydrophobic regions, was generated using Maestro. The preferential binding of ligand hydrophobic groups in these regions can enhance receptor-binding affinity. The contour surface was plotted at a level of −1.40 kcal/mol.

## 3. Results

### 3.1. Nuclear receptor binding-site flexibility

Nuclear receptors such as the GR and the PR are known for their ability to adopt large conformational changes to accommodate structurally diverse ligands. In many cases, they bind agonists and antagonists with distinctive conformations. We superimposed all available GR-LBD agonist-bound structures to get a preliminary idea of the scope of binding-site flexibility. The two GR-LBD/Dex complex structures obtained from the PDB were solved independently by two separate groups under slightly different conditions. The 1m2z structure has two chains in the asymmetric unit, while the 1p93 has four. The GR-LBD/deacylcortivazol structure, 3bqd, has one chain in its asymmetric unit. The seven chains were extracted from the PDB structures and aligned using 1p93 chain A as template. Fig. 2 shows the overlay of these structures. The protein backbone among the structures aligns very well, and its movement around the agonist binding site is limited. Receptor side-chain movements, on the other hand, are relatively extensive; many side chains that line the binding site can adopt several conformations, particularly those residues forming the 17α pocket.

The flexibility of nuclear receptor ligand-binding sites is further demonstrated by crystal structures of the PR-LBD in complex with two different ligands: progesterone in 1a28 and MF in 1sr7. Fig. 3 shows the overlay of chain A from each structure. The backbone of the two structures aligns well, with only minor differences in two loop regions. However, extensive side-chain rearrangements created a large 17α pocket in the binding site of the MF-bound 1sr7 structure that was not seen in the progesterone-bound 1a28. This pocket is crucial to accommodate MF, which has a large furoate group at this position. This example further demonstrates that side-chain conformational changes in agonist-bound nuclear receptors are sufficient to cause significant changes in the shape of the binding site without major backbone conformational changes. Together, these examples show that it is essential to explore the complete side-chain conformational space to get a realistic representation of ligand–receptor interactions for new GCs. However, it appears that only limited sampling of backbone conformations is necessary for modeling.

### 3.2. Validation of the IFD protocol

Before using the IFD protocol to model the binding of new glucocorticoids, we carried out two validation studies to test whether the protocol can reproduce the experimental crystal structures of two related nuclear receptors. In the first exercise, our objective was to assess the ability of IFD to maintain the observed crystal structure. For this purpose, we carried out an IFD of Dex with the GR-LBD structure 1p93 (chain A). Even though Dex does not occupy the 17α pocket, this pocket does exist in the crystal structure, and this exercise allowed us to investigate whether IFD

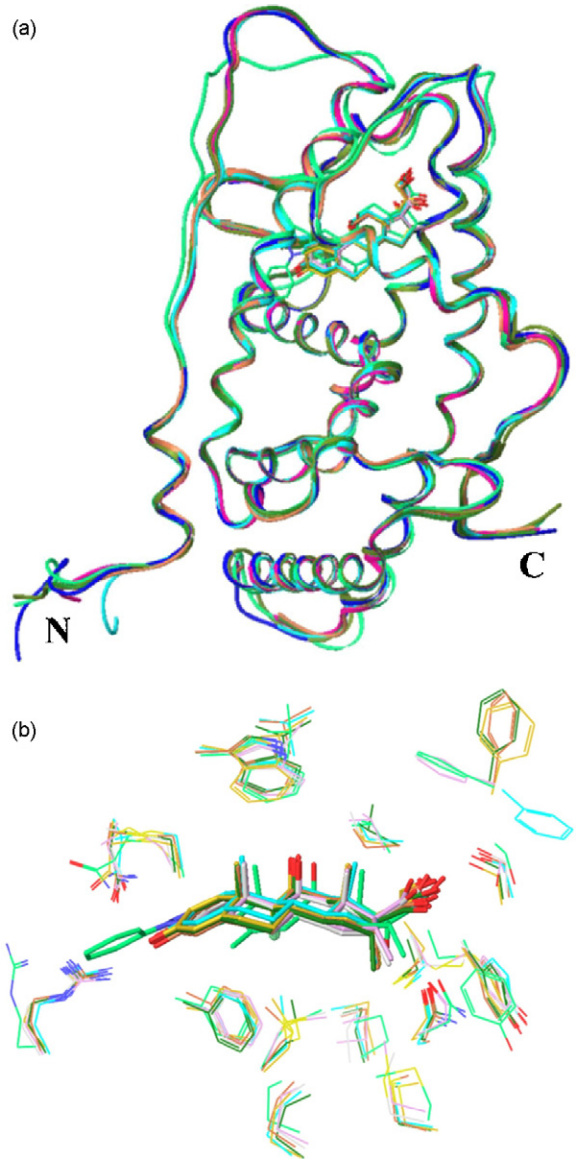


**Fig. 2.** Overlay of all chains in the three available agonist bound GR-LBD structures (1m2z:AB; 1p93:ABCD; 3bqd). O atoms are colored red, N blue, S yellow, F light green and Cl green throughout this paper. Carbon atoms are colored by molecules. (a) The backbone conformation is consistent among the structures. Each colored ribbon represents a unique chain from the crystal structures (cyan: 1p93A; brown: 1p93B; green: 1p93C; yellow: 1p93D; pink: 1m2zA; blue: 1m2zB; lime green: 3bqd). (b) Side-chain flexibility around the ligand-binding site. Side chains with at least one atom within 5 Å of the ligand are displayed (some residues were removed for clarity).

would cause it to artificially collapse. As shown in Fig. 4, IFD closely reproduced the Dex-bound GR-LBD crystal structure, and no major distortions in side-chain conformations were generated. When the best-ranked IFD structure is overlaid on its crystal counterpart using the Cα atoms, the resulting RMSD is only 0.12 Å. When limited to the binding site, the RMSD for the backbone heavy atoms of residues within 5 Å of the docked Dex is 0.21 Å, while the RMSD for the side-chain atoms of these residues is 1.17 Å. Most importantly, the 17α pocket does not collapse and the general shape of the ligand-binding site is preserved.

For the second IFD validation study, we used PR-LBD structures since we could not find an appropriate example from the limited number of available GR-LBD crystal structures. The LBD of PR, which also belongs to the glucocorticoid-like nuclear-receptor family, shares 54% sequence identity with the GR-LBD. While there is no 17α pocket in the receptor binding-site when PR-LBD binds to its endogenous ligand progesterone (*i.e.*, 1a28), the pocket is created when the protein is bound to MF, as in 1sr7. Since it would therefore be impossible to find a binding pose for MF in the 1a28 structure using rigid docking, we assessed whether a reasonable pocket would be generated when using IFD to model MF to 1a28.

As seen in Fig. 5, the IFD protocol did indeed induce a 17α pocket in the PR-LBD binding site. The model of MF binding to PR-LBD obtained by IFD is very similar to the crystal structure of 1sr7, including side-chain conformations. Since there is a gap between Asn705 and Pro708 in the PR-MF crystal structure, the top-ranking IFD structure was superimposed on the 1sr7 structure using Cα atoms of residues 712–930. The RMSD of the Cα atoms of the IFD structure compared to the crystal structure is only 0.32 Å. In the binding site, the RMSD of the backbone atoms for the residues within 5 Å of the docked MF is 0.40 Å, while the RMSD for the side-chain atoms of these residues is 1.35 Å. Furthermore, the RMSD for MF heavy atoms is only 1.13 Å when the protein Cα atoms are superimposed. The volume of the PR-LBD binding site in the crystal structures increases from 493 $Å^3$ when progesterone is bound to 635 $Å^3$ when MF is bound. In comparison, the binding site of the PR-LBD/MF structure generated by IFD has a volume of 623 $Å^3$. Additionally, IFD of another marketed glucocorticoid with a 17α furoate group, FF, shows that this molecule can also induce a similar 17α pocket in the PR-LBD binding site (data not shown). This agrees with a recent experimental finding that FF has subnanomolar affinity for PR ($pEC_{50}$ = 9.05) [38].

These two validation studies demonstrate that IFD is capable of both maintaining the experimental structure and inducing a reasonable binding pocket in nuclear receptors in the presence of a larger ligand. By exhaustively sampling the conformational space for protein side-chains involved in the binding of ligand molecules, the IFD protocol can cover many accessible receptor binding-site configurations. At the same time, limited backbone flexibility was explored, through constrained energy minimization. In the following sections, we report our modeling results for two potent GCs with large 17α groups, MF and des-CIS.

### 3.3. Modeling of MF

Mometasone furoate is the first marketed steroid to incorporate a furoate group at the 17α position to achieve a very high affinity for GR (Table 1). No crystal structure or computer model for this compound in complex with the GR-LBD has been reported. A rigid docking of MF to the GR-LBD was carried out using the 1p93 chain A with a vdW scaling of 0.8 for both the protein and ligand non-polar atoms. In this model, MF fits at the binding site with the furoate group docked into the 17α pocket. The typical hydrogen-bonding patterns seen in the crystal structure of Dex are maintained in this rigid docking model, *e.g.*, the C3-carbonyl group with Gln576 and Arg611, and the 11β-hydroxyl group with Asn564. But, many short vdW contacts (defined as when the distance between a pair of non-bound atoms is less than 80% of the sum of their vdW radii) are observed between the furoate group and the protein side-chain atoms in the 17α region. Most of the steric conflicts came from the close contacts of MF with two Met residues (Met560 and Met601). The long flexible side chains of these amino acids make them prone to adopting different conformations that can enlarge the pocket observed in the crystal structure. Hence, we would expect GR to adopt conformational changes to better accommodate the large 17α furoate group of MF.

An IFD was then carried out using the same protein structure to get a more realistic picture of MF and GR-LBD interactions. Conformations of side chains within 4 Å of the docked ligand were

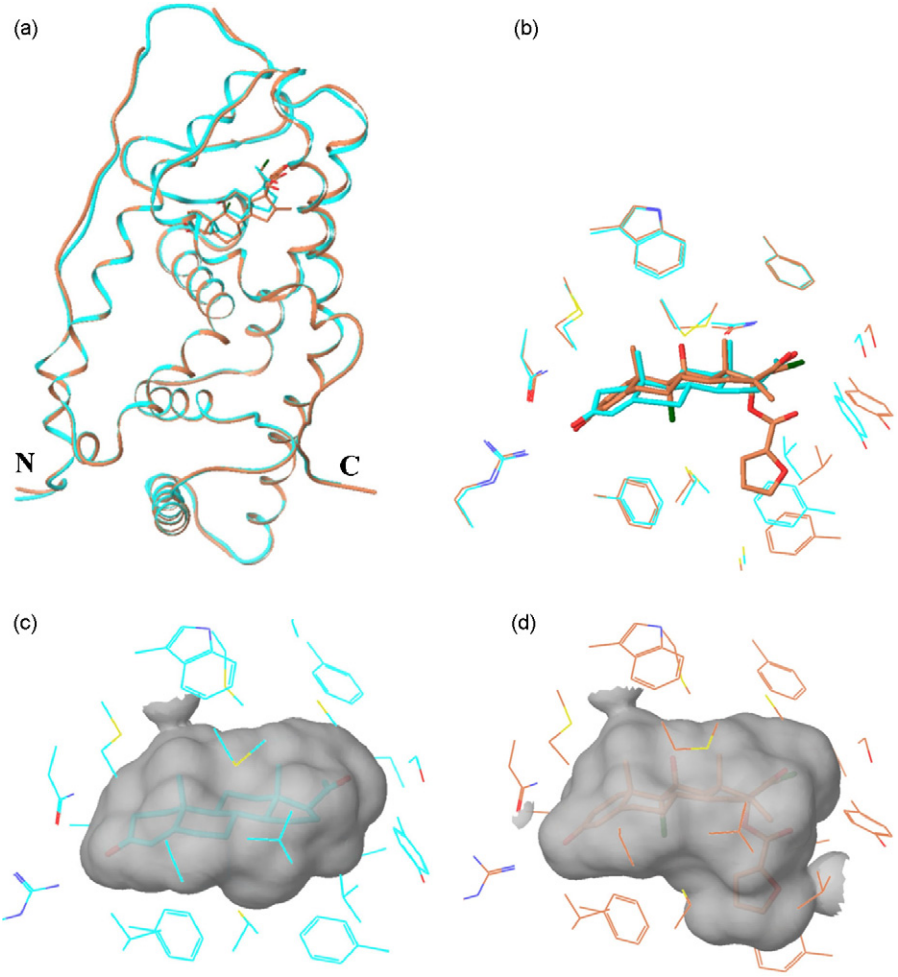


**Fig. 3.** Comparison of two PR-LBD crystal structures (PDB IDs: 1a28 and 1sr7). Carbon atoms of 1a28 are colored cyan, and C atoms of 1sr7 brown. (a) Overlay of the two PR-LBD structures shows minimal backbone conformational changes. (b) Large side-chain conformational changes observed in the ligand-binding site. Residues with at least one atom within 5 Å of the corresponding ligand are displayed (some residues were removed for clarity). (c) The shape of the binding site in the progesterone-bound structure. (d) The shape of the binding site in the MF-bound structure. A large 17α pocket was induced in this structure to accommodate the furoate group.

exhaustively sampled during IFD, but only limited backbone flexibility was allowed by subjecting the protein to energy minimization during the first two stages of the IFD process. As mentioned in the Methods section, there is a water cavity next to the binding site behind the Gln570 and Arg611 residues. Since water molecules were not included in the IFD and the steroid core of MF is the same as that of Dex, we kept the conformation of these two residues fixed so that no artificial conformational changes would be generated.

Fig. 6 shows some of the important structural features observed in the GR-LBD/MF model generated by IFD. First, the short vdW contacts seen in the rigid-docking model no longer exist. The two Met residues that produced most of the bad vdW contacts in the rigid-docking model adopt a different conformation, moving away from MF and creating a slightly expanded 17α pocket. The overall protein structure is very similar to the crystal structure, with the RMSD of Cα atoms only 0.12 Å. The RMSD of backbone heavy atoms of all residues within 5 Å of the docked MF molecule is 0.20 Å, while the RMSD of the side-chain heavy atoms for these residues stands at 1.25 Å. Thus, the change in the binding site that occurs with IFD mainly results from changes in side-chain conformations. The MF structure in the IFD model is much less strained compared with that in the rigid model, *i.e.*, its conformational energy in the IFD model is 6.8 kcal/mol lower. The IFD-derived model of MF bound to GR allows for more accurate interpretation of the key binding interactions as well as for how the GR-LBD reacts to the binding of this agonist.

As shown in Fig. 6(a), MF makes several hydrogen-bond interactions with the GR-LBD: the C3-carbonyl group of MF with the two conserved residues Glu570 and Arg611, the 11β-hydroxyl group and Asn564, and the C20-carbonyl oxygen and Thr739. These polar interactions, which resemble those in the crystal structures of GR bound with Dex, contribute to MF binding affinity and specificity for the GR. At the same time, the steroid core maintains close contacts with the binding pocket through vdW and hydrophobic interactions. The direct interactions of MF with two hydrophobic residues, Ile747 and Phe749, in the loop preceding the AF-2 helix as well as with Leu753 at the beginning of this helix contribute to helix stabilization and ensure the receptor is locked in the agonist conformation. The 17α furoate group of MF fits well into the slightly enlarged side pocket enabled by the side-chain movements of three residues in this region, Met560, Leu563, and Met646, as shown in Fig. 6(b).

Fig. 6(c) highlights the hydrophobic interactions between MF and the receptor binding-site. The receptor hydrophobic contour map shows that four regions in the binding site strongly prefer ligand hydrophobic groups. In the GR-LBD/MF model, the first hydrophobic region is occupied by the A-ring of the steroid core. A

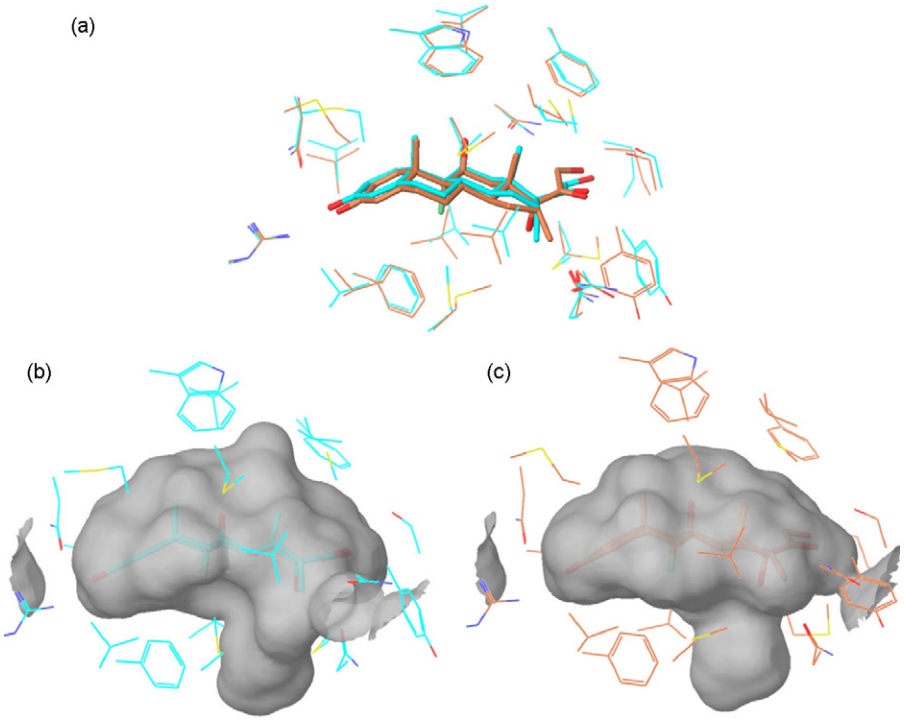


**Fig. 4.** Comparison of the GR-LBD/Dex crystal structure (1p93 chain A) and the top-ranked IFD model. C atoms in the crystal structure are colored cyan, and C atoms in the IFD structure brown. (a) The protein conformation is preserved in the IFD structure with no major distortion in side-chain conformation of the binding-site residues. (b) Shape of the binding site in the GR-LBD/Dex crystal structure. (c) Shape of the binding site in the top-ranked IFD structure. The 17α pocket is preserved even though Dex does not occupy this pocket.

second region is located closed to the 16α position and is occupied by a methyl group from MF. The third major hydrophobic region is located at the lower part of the 17α pocket, which is nicely occupied by the 17α furoate group. The furoate group therefore plays a crucial role in enhancing the binding affinity of MF to GR. In the GR-Dex crystal structure, a C21-hydroxyl group makes weak hydrogen bonds with receptor residues Thr739 and Asn564. Mometasone furoate, on the other hand, has a chlorine atom at this position that is hydrophobic in nature and consequently cannot form these hydrogen bonds. But, as shown in the hydrophobic map, the MF C21 chlorine sits in a small hydrophobic region sandwiched between the two polar residues and makes direct contacts with the hydrophobic side chains of Met560, Ile747, and Phe749. This gain in hydrophobic interaction energy probably offsets the loss in polar interactions. Thus, the higher affinity of MF for GR can be readily accounted for by interactions between the receptor and the furoate group and by the hydrophobic interactions at the C21 chlorine.

### 3.4. IFD of des-CIC

des-CIC has a larger substituent at the 16,17α positions than MF, and the 17α pocket of GR must undergo even greater expansion to accommodate this compound. Rigid docking with the available GR-LBD crystal structures did not produce any reasonable models. We therefore carried out an IFD calculation with des-CIC to further explore the "soft" nature of this pocket as seen in the MF IFD study.

Fig. 7 shows the binding site of the best-ranking IFD model for des-CIC. Analogous to Dex and MF, the C3-carbonyl and 11β-hydroxyl groups of this molecule make hydrogen bonds with the Gln570, Arg611, and Asn564 residues, thereby maintaining the typical polar interaction network. The C21-hydroxyl group hydrogen bonds with Thr739 in a similar way as Dex. Again, the overall protein structure does not change significantly: the RMSD of protein Cα atoms is only 0.12 Å compared with the starting crystal structure. The RMSD of backbone heavy atoms of all residues within 5 Å of the docked des-CIC molecule is 0.19 Å, while the RMSD of the side-chain heavy atoms for these residues is at 1.25 Å. These RMSD values are almost identical to those of the MF IFD structure.

Further expansion of the 17α pocket was observed in the des-CIC bound IFD model, with such expansion occurring mainly by deepening the bottom of the hydrophobic subpocket compared with those of both the Dex crystal structure and the MF IFD model. As shown in Fig. 7(b), the additional pocket expansion is made possible primarily by the conformational changes of four binding site residues: Phe623, Leu621, Met646, and Cys643. Phe623 is located just below the steroid A and B rings. In the des-CIC IFD structure, this residue shifts upward and slightly elevates the steroid core, most likely due to the lack of a chlorine atom at the 9α position compared with MF. By moving this residue upward, the receptor can optimize its contact surface with the steroid core and avoid the creation of an unfavorable void. The reorganization of the other three residues, which are located at the bottom of the 17α pocket, created a larger and deeper subpocket in the binding site that can better accommodate the hydrophobic cyclohexyl group.

## 4. Discussion

In this study, we report the modeling of GCs with large 17α groups in the GR-LBD using an IFD approach. We first validated the accuracy of the IFD method using known crystal structures of two steroid nuclear receptors, GR-LBD/Dex and PR-LBD/MF complexes. The validation studies demonstrated that this approach is appropriate for modeling nuclear receptors in two ways: it maintains the non-occupied 17α pocket in the GR-LBD/Dex structure, and it properly induces a 17α pocket when it is lacking in the starting PR structure. In both cases, the induced-fit model

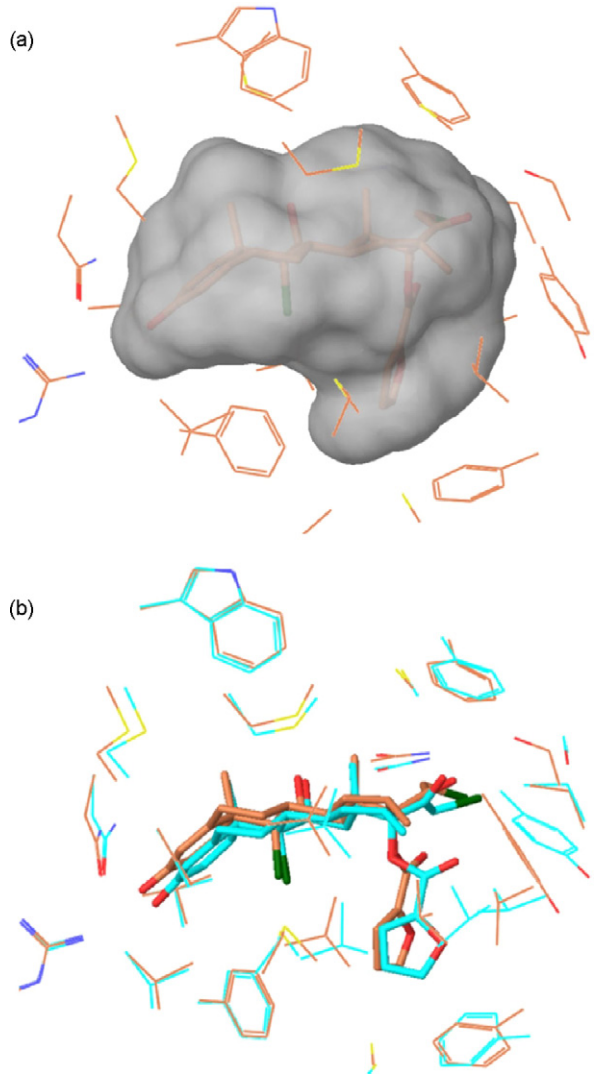


**Fig. 5.** Comparison of the PR-MF crystal structure (1sr7) and the top-ranked IFD model. C atoms in the crystal structure are colored cyan, and C atoms in the IFD structure brown. (a) A 17α pocket was induced in the IFD model. The model was generated with the progesterone-bound PR-LBD structure (1a28) where there is no 17α pocket. The pocket was created mainly by side-chain conformational changes in the 17α region with minimal backbone involvement. (b) Overlay of the binding site of 1sr7 and the IFD model showing that the IFD model closely reproduced the crystal structure.

closely reproduces experimental structures, with all key interactions characteristic of the crystal structures preserved and the RMSD of protein Cα atoms within 0.4 Å. Although rigid docking has been quite successful for modeling many protein–ligand interactions, this method fails to produce accurate structural information when ligand binding requires conformational changes in the target protein. Thus, protein flexibility must be taken into account in order to get a true picture of protein–ligand interactions. This

**Table 1**
Relative GR binding affinity reported in the literature for the compounds studied.

| Compound | RBA | Reference |
|---|---|---|
| Dexamethasone | 100[a] | |
| Mometasone furoate | 1200–2900 | [23–26] |
| Ciclesonide | 12 | [27] |
| Desisobutyryl-ciclesonide | 1212 | [27] |

[a] Dexamethasone used as standard in calculating relative binding affinity.

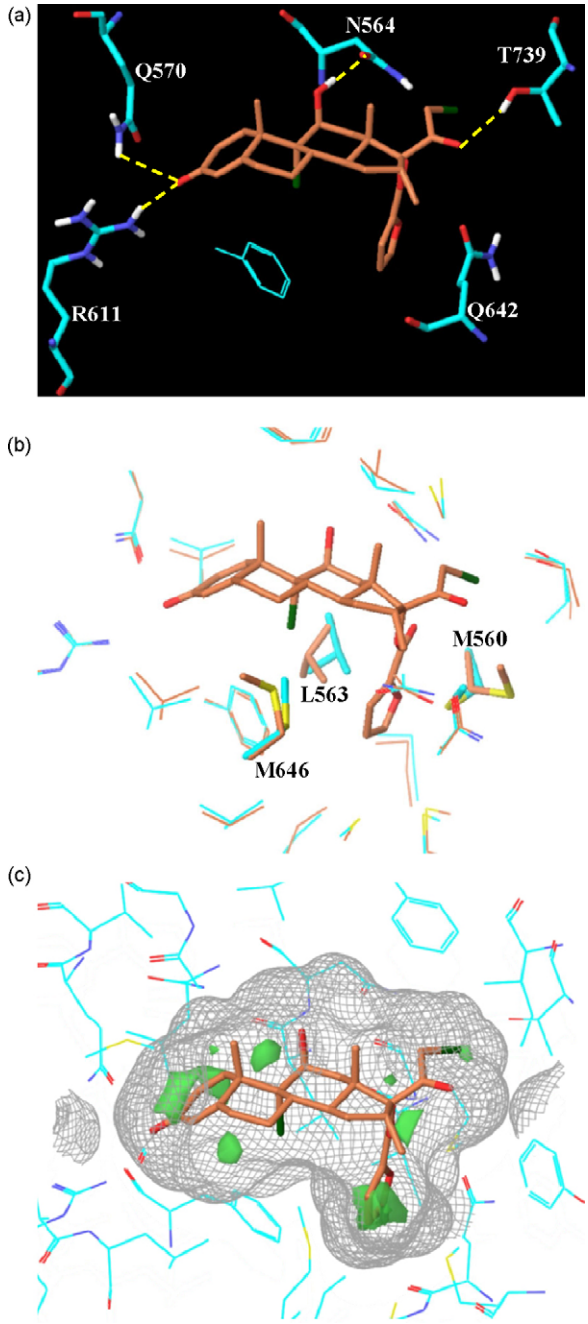


**Fig. 6.** IFD model of MF binding to GR-LBD. (a) Polar interactions between MF and GR-LBD. Hydrogen bonds formed by the C3-carbonyl, C11-hydroxyl, and C20-carbonyl groups are highlighted. C atoms of GR-LBD are colored cyan, and C atoms of MF brown, H atoms white. (b) Overlay of the IFD model with the starting crystal structure (1p93 chain A) showing conformational changes in Met560, Leu563, and Met646 expand the 17α pocket. C atoms in the crystal structure are colored cyan, and C atoms in the IFD structure brown. (c) Hydrophobic map of the GR-LBD binding site. The major hydrophobic regions (green) are occupied by the steroid core, 17α furoate group, and the C21-Cl atom of MF. The 17α furoate group of MF fits well in the 17α pocket. C atoms of GR-LBD are colored cyan, and C atoms of MF brown.

requires sampling of the side-chain flexibility of at least the binding-site residues during docking. The IFD approach we used adequately samples this flexibility to generate more accurate poses and reasonable binding structures. Moreover, limited backbone flexibility is accounted for by energy minimization of the protein structure.

Recent structural studies of steroid nuclear receptors, *i.e.*, the mineralocorticoid receptor, the progesterone receptor, and the

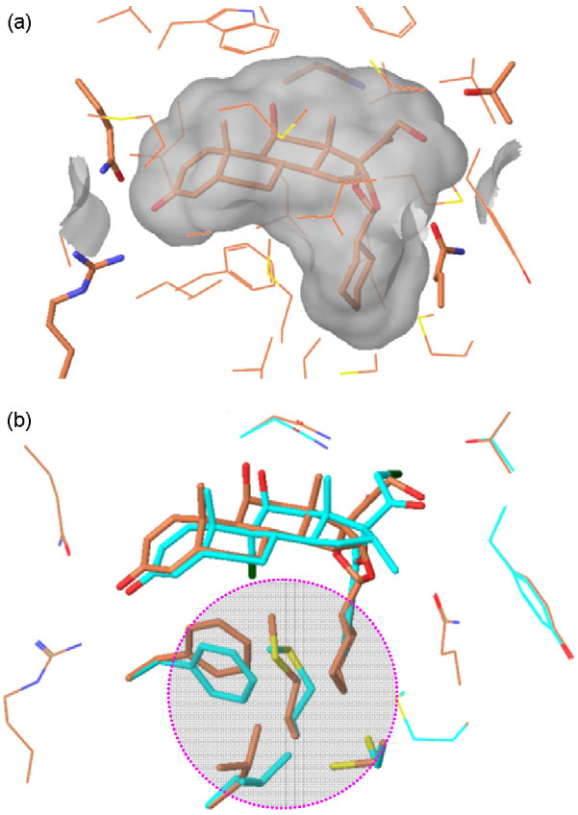


**Fig. 7.** IFD model of des-CIC binding to GR-LBD. (a) The GR-LBD binding pocket in the IFD structure. The cyclohexylacetal moiety occupies an expanded 17α pocket. (b) Overlay of the IFD models of MF and des-CIC. The further expansion of the 17α pocket is made possible by conformational changes of residues Leu621, Phe623, Cys643 and Met646. C atoms in the MF IFD model are colored cyan, and C atoms in the des-CIC IFD model are brown.

androgen receptor, reveal a great range of conformational flexibility in their ligand-binding pockets [10,39–41], indicating the adaptability of nuclear receptors in general. These receptors all have multiple Met residues in their binding sites whose conformational changes, in combination with occasional flipping of a Trp side-chain, can accommodate many variations in the C6, C17α, and C18 positions of the steroidal core. GR and PR share high sequence identity (54%) and many structural similarities in their ligand binding domains. While the natural ligand of PR, progesterone, has a hydrogen atom at the 17α position, molecules with larger groups at this position have been known to bind with this receptor. Prior work using crystallographic analysis of the PR-LBD revealed that this receptor accommodates larger moieties, such as the furoate group of MF, by adoption of alternative side-chain rotamer conformations of ligand-proximal amino acids [31]. The PR-LBD backbone, in contrast, does not need to undergo marked changes to make room for different ligands, as the inherent flexibility of the side chains in the binding pocket is sufficient. This may be true for GR as well, because no significant backbone distortions were observed among the limited number of experimental structures (agonist form), while large side-chain conformational variations were observed even in crystal structures of the same ligand. In a recent example, the size of the GR binding site was shown to change dramatically upon the binding of deacylcortivazol with only minimal backbone conformational changes [10].

In light of the above observations, the IFD protocol seems especially useful for studying GR, whose conformational adaptability to ligand binding is well documented in the literature. The GR-LBD is not stable in the apo form, cannot be crystallized without the presence of a co-activator peptide, and in most cases requires co-expression to get stable crystals. To date, only three GR crystal structures in complexes with GCs and one with a GR antagonist are available in the public domain. The binding site in the agonist-bound form is closed and its size fails to account for the binding of a large number of steroidal and non-steroidal ligands reported in the literature. Induced-fit docking allows for the expansion of the GR binding site and docking of larger GCs. The models generated using this method can provide critical insight into receptor protein flexibility, the mechanisms of ligand–receptor interactions, and a structural template for designing novel GCs. Furthermore, GR conformational adjustment upon binding of different ligands may lead to changes in affinities for different cofactors and in the dimer association equilibrium; understanding these changes is therefore essential for achieving desired therapeutic and safety profiles.

Using IFD based on GR-LBD/Dex crystallography, the key binding interactions between MF and GR were determined. These include hydrogen bond interactions between the C3-carbonyl group of MF and Gln570 and Arg611 of the GR, the C11-hydroxyl group and Asn564, and the C20-carbonyl group and Thr739. These interactions were observed in the crystal structures of other GCs, and maintaining this interaction network is crucial for the recognition of MF by the GR-LBD. In addition, we identified strong lipophilic interactions between the C17 furoate ester and the 17α pocket, and a lipophilic interaction between the C21 chlorine and the receptor. As shown in our examples, the GR 17α pocket demonstrates a significant degree of flexibility to accommodate ligands, while the protein backbone remains fairly rigid.

Our findings verify the close association of the furoate moiety within the 17α pocket of GR and the ability of the receptor to conform and bind tightly to this group. A greater understanding of the GR over recent years has led to the development of compounds designed specifically to fit closely into the binding site, including incorporation of the furoate moiety, which appears to greatly enhance the molecular interactions between the corticosteroid and the receptor. Corticosteroid potency is measured via techniques such as the McKenzie assay, and MF has been consistently ranked as having one of the highest potencies using these methods [42,43]. Another commercial corticosteroid, FP, has recently been modified through substitution of a furoate ester (FF) in an effort to improve its pharmacologic profile. Like MF, FF also demonstrates tight binding within the 17α pocket of the GR [44].

Our additional IFD study using des-CIC as the ligand provided further evidence for the flexible nature of the GR binding site and the 17α pocket specifically. Compared with MF, des-CIC has an even larger substituent at the 17α position, and the IFD model provided evidence for expansion of the 17α pocket to accommodate it. Receptor flexibility was achieved through rearrangements of several binding-site residues, while the protein backbone remained rigid. GR is the only glucocorticoid-like nuclear receptor that has a stable and well-defined 17α pocket, though the induction of this pocket by various ligands has been observed in other receptors of this family. Strong binding of a ligand to this pocket may not only increase the affinity of the molecule for GR but could potentially improve its selectivity over other nuclear receptors as well.

The binding-site similarities between GR and other nuclear receptors, including PR, raise logical concerns about undesired effects of GC administration. However, despite the ability of MF to bind with PR under *in vitro* conditions, there is little evidence for any significant clinical ramifications of this association. This may be a result, in large part, of the negligible systemic absorption observed with MF, which has an oral bioavailability of $<0.1\%$ and

undergoes almost complete first-pass metabolism [45]. Even when administered at 20 times the recommended dose for treatment of allergic rhinitis, MF administered intranasally was not shown to adversely affect urinary free-cortisol or plasma levels, nor was there evidence of hypothalamic–pituitary–gland axis suppression [46].

## 5. Conclusions

In this paper, we report the modeling of glucocorticoids with large 17α group, MF and des-CIC, to GR-LBD using an IFD protocol. With the limited number of available crystal structures, the large 17α groups make it hard to model these compounds using rigid docking. Close examination of the available agonist-bound crystal structures of the steroid nuclear receptors shows that their binding site is very flexible, and the change in size and shape of the binding site comes mainly from the side-chain conformational changes. Our validation studies show that the IFD protocol is appropriate for the study of GR and other nuclear receptors. The MF IFD model highlights the crucial molecular features for its high GR affinity, *i.e.*, the hydrogen bonding network and especially the favorable hydrophobic interactions between the furoate group and the GR 17α pocket. IFD model with des-CIC demonstrates the ability of the 17α pocket to expand even further to accommodate ligands with bulkier groups. In both models, the protein side-chains in this pocket provide the flexibility to adapt to the ligands, while the backbone demonstrates minimal movement. Our findings clearly demonstrate the benefits of IFD techniques for structural modeling when receptor conformational change is required.

## Acknowledgement

The authors thank Adelphi Inc. for editorial support.

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