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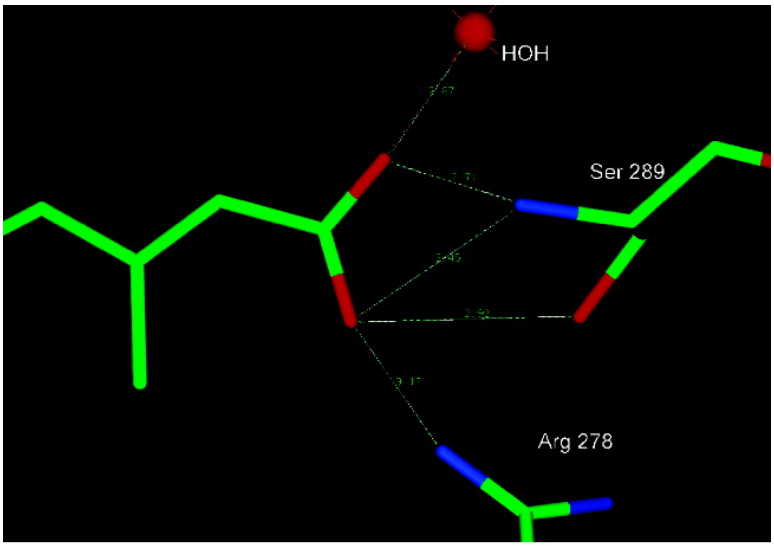

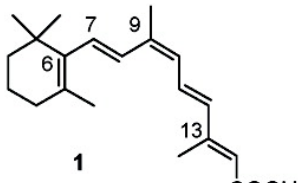

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9-*cis*-retinoic acid

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Ligand Recognition by RAR and RXR Receptors: Binding and Selectivity

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Fundamental biological functions, most notably embriogenesis, cell growth, cell differentiation, and cell apoptosis, are in part regulated by a complex genomic network that starts with the binding (and activation) of retinoids to their cognate receptors, members of the superfamily of nuclear receptors. We have studied ligand recognition of retinoic receptors (RXRα and RARγ) using a molecular-mechanics-based docking method. The protocol used in this work is able to rank the affinity of pairs of ligands for a single retinoid receptor, the highest values corresponding to those that adapt better to the shape of the binding site and generate the optimal set of electrostatic and apolar interactions with the receptor. Moreover, our studies shed light onto some of the energetic contributions to retinoid receptor ligand selectivity. In this regard we show that there is a difference in polarity between the binding site regions that anchor the carboxylate in RAR and RXR, which translates itself into large differences in the energy of interaction of both receptors with the same ligand. We observe that the latter energy change is canceled off by the solvation energy penalty upon binding. This energy compensation is borne out as well by experiments that address the effect of site-directed mutagenesis on ligand binding to RARγ. The hypothesis that the difference in binding site polarity might be exploited to build RXR-selective ligands is tested with some compounds having a thiazolidinedione anchoring group.

Introduction

Retinoids, vitamin A and its natural and synthetic analogues, are a very important group of hormones that regulate a wide variety of biological functions through a mechanism that entails their binding to two subfamilies of nuclear receptors (NRs) known as RXRs and RARs as the first step in a series of processes that lead to gene transcription.[1] Each of the nuclear receptor subfamilies (RAR and RXR) has three isotypes known as α, β, and γ.[2,3] These receptors are involved in such important functions as embryogenesis, cell growth, and cell differentiation. Their far-reaching biological effects have motivated the search for RAR- or RXR-selective agonists and antagonists as drug leads for dermatology, oncology, etc.[1,4] The cognate ligand of RXR is 9-*cis*-retinoic acid (**1**), a molecule that also binds and transactivates RAR with very similar affinity and efficiency. On the other hand, *all-trans*-retinoic acid (**2**), the cognate ligand of RAR, does not bind to the RXR receptor.[5,6]

Until recently, the design of novel retinoid receptor modulators has been carried out by ligand-based structure−activity relationship protocols.[7] The structural determination of the ligand binding domain (LBD) of the unbound RXR and of the LBDs of RARγ and RXRα bound to a variety of ligands opened the door to an alternative receptor-based search for potent and/or selective agonists.[8] Knowledge of the three-dimensional structure of these complexes offers the opportunity to analyze the energetic contributions to binding in these receptors, providing a rational basis for the design of novel ligands. Here we present a docking protocol for the binding of agonists to RXR and RAR. The docking protocol reproduces the ranking of the affinity of selected ligands for retinoic receptors, a list which includes the cognate retinoid ligands for RXR and RAR, some 9-*cis*-locked, cyclopropane-based analogues,[9] and retinoids containing a stilbene hydrophobic moiety and their *o*-methyl derivatives.[7,10,11] Particularly, it is shown that the ligand−receptor interaction energy profiles for pairs of structurally related analogues are a good gauge for their ranking of ligand affinity for the retinoid receptors. We demonstrate that receptor−ligand shape complementarity is of the essence in the design of optimal ligands.

Receptor-selective ligands are a high priority in the search for NR-based drug leads, since native NR ligands present systemic side effects and toxicity due to their lack of binding specificity, especially at high concentrations. For instance, nonselective retinoid ligands when employed as drugs have side effects such as teratogenicity and mucocutaneous toxicity,[7] which are significantly reduced when specific RXR agonists are used.[10] Furthermore, it has been shown that tumor-specific apoptosis can be driven by RXR-selective agonists,[4b] like in the case of prostate malignancies, and by pan-agonists and RARγ-selective agonists, for pancreatic cancer cells.[4c] Finally, selective RXR agonists may offer an alternative approach for the treatment of metabolic disorders such as type II diabetes and obesity.[11] Some RAR-isotype-selective modulators and RXR-selective ligands have been recently reported.[9−13] To understand receptor selectivity, we have analyzed the change in the

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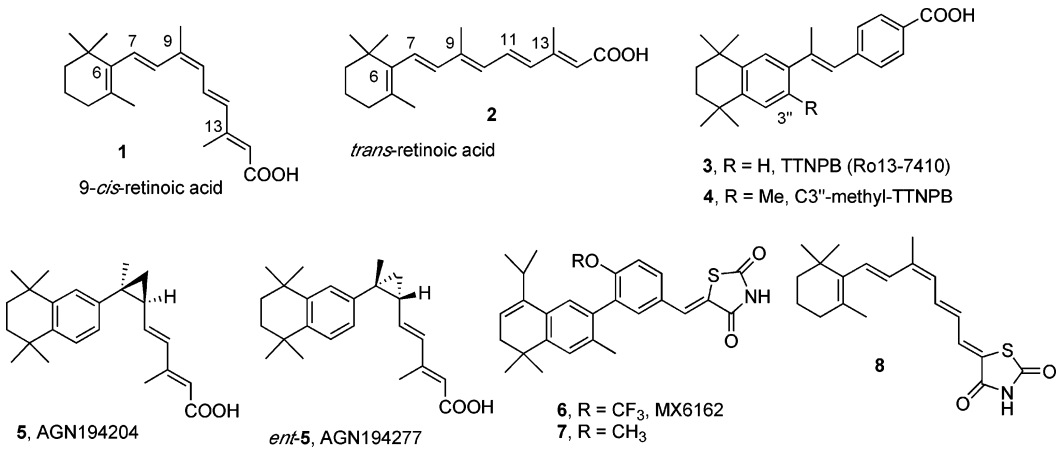

Figure 1. Structure and nomenclature of the analogues studied in this work.

receptor−ligand interaction and the desolvation energy components upon ligand binding to RXR and RAR. The calculated ligand−protein interaction energy profiles for a single agonist exhibit a large energy gap between the RAR and RXR complexes, which can be traced back to the higher polarity of the ligand anchor region in the binding pocket of RAR as opposed to RXR. The above-mentioned energy disparity is almost completely canceled off by the addition of the binding solvation energy penalty term to the free energy predictor function. The interaction energy−desolvation energy compensation is supported by mutagenesis experiments of the RAR binding site. The difference in polar residue content around the ligand's anchoring group was therefore regarded as a discriminating factor leading to the design of selective RXR ligands, and the possibility that analogues with anchoring groups that differ in chemical structure from a carboxylate could fulfill this paradigm was considered. To explore this idea, we evaluated the binding of two thiazolidinedione-based analogues, differing in the bend between the hydrophobic moiety and the anchoring fragment, to RXR and RAR. These kind of analogues have generated interest as potential RXR-specific antagonists.[14] Our results indicate that the inclusion of a kinked shape in the designed ligand is vital in guiding the anchoring moiety to an optimal set of interactions with polar groups of RXR while reducing the interactions of the carboxyl group surrogate with the polar residues of RAR.

Methods

The binding of a series of agonists listed in Figure 1 to RXRα and RARγ was modeled after the crystallographic structure of RXRα bound to 9-*cis*-retinoic acid (**1**) (PDB entry 1FBY),[8d] the complex formed by RARγ and *all-trans*-retinoic acid (**2**) (PDB entry 2LBD),[8b] and the complex between RARγ and **1** (PDB entry 3LBD).[8f] Hydrogen atoms were added to these receptors, using the Biopolymer module in InsightII.[15] The docking of the ligands listed in Figure 1 to both RXRα and RARγ receptors was carried out by the following protocol.

Initial Ligand "Docking". The LIGANDFIT module of Cerius2 was used to dock the ligands shown in Figure 1 into RARγ and RXRα.[16] This protocol generated four initial ligand orientations, which were reduced by half by selecting those orientations that brought the ligand polar moiety closer to the receptor's anchoring groups (e.g., Arg 316 in RXRα and Arg 278 in RARγ).

Molecular Dynamics Simulation of the Complex. The resulting complexes underwent a 100 ps molecular dynamics (MD) simulation at low temperature (200 K), using the CVFF[17] potential force field in the molecular mechanics Discover software suite. The equilibration stage lasted 20 ps, while the production stage lasted 80 ps. All residues beyond a 6 Å radius from the ligand were held fixed during the MD simulations.

Analysis. The energetics of binding was analyzed with the help of two algorithms. The first includes only the ligand−receptor interaction energy (ΔG_{inter}), which can be written in its usual form as

$$\Delta G_{\text{inter}} = \sum_{i,j} \frac{A_{ij}}{r_{ij}^{12}} - \sum_{i,j} \frac{B_{ij}}{r_{ij}^{6}} + \sum_{i,j} \frac{q_i q_j}{\epsilon_{ij} r_{ij}} \qquad (1)$$

where the first two sums correspond to the Lennard-Jones-type van der Waals contribution to the interaction energy, while the last sum is a Coulombic-type term that depicts the electrostatic contribution to the interaction energy. r_{ij} is the distance between atoms i and j of the ligand and receptor, respectively, A_{ij} and B_{ij} are constants, q_i and q_j are the charges of the ligand and receptor, and ϵ_{ij} is a dielectric constant, which in our calculations was set to 1.

The second protocol incorporates the solvation contribution brought about by binding (ΔG_{solv}), and can be written as

$$\Delta G_{\text{bind}} = \Delta G_{\text{inter}} + \Delta G_{\text{solv}} \qquad (2)$$

To evaluate the contributions to eq 2, MD frames from the last trajectory with a 2 ps spacing were selected. For each frame the energy of interaction between the ligand and the receptor and the desolvation term in eq 2 were computed. The first term was calculated using the DECIPHER module in InsightII. We have followed a standard protocol for the evaluation of ΔG_{solv} in eq 2, which partitions it into the sum of its polar ($\Delta G_{\text{solv,pol}}$) and hydrophobic ($\Delta G_{\text{solv,hydro}}$) components. The polar solvation term was calculated by the DelPhi protocol, an approach that solves the linearized Poisson−Boltzmann equation by a finite differences algorithm.[18] We used for these calculations the default parameters provided by the Solvation module[15] in InsightII for the kind of force field used in these calculations, with the accuracy level set to regular, the internal dielectric constant set to 1, and the ionic strength set to 0. The hydrophobic solvation component was calculated by a term proportional to the accessible surface area (A), which can be written as

$$\Delta G_{\text{solv,hydro}} = \gamma A + b$$

where γ and b are constants. The values of these parameters used in this work are the ones that are provided by the InsightII Solvation module[15] for the kind of force field used in this work.

The binding selectivity can be estimated from the calculated difference in the predicted free energy of ligand binding to the

Table 1. Experimental Dissociation Constants for Selected Ligands[a]

ligand name	$K_{d,RXR\alpha}$ (nM)	$K_{d,RAR\gamma}$ (nM)	ligand name	$K_{d,RXR\alpha}$ (nM)	$K_{d,RAR\gamma}$ (nM)
9-*cis*-retinoic acid, **1**	1.5	0.8	AGN-194204, **5**	0.4	>30000
all-trans-retinoic, **2**	NAA	0.2	AGN-194277, *ent*-**5**	60.0	>10000
TTNPB, **3**	NAA	26.0	MX6162, **6**	NA	NAA
*C*3″-MeTTNPB, **4**	32.0	645.0			

[a] NAA = not an active agonist. NA = not available.

two receptors ($\Delta\Delta G_s$), which in principle can be written as

$$\Delta\Delta G_s = \Delta\Delta G_{bind} + \Delta\Delta G_{a\rightarrow h} \quad (3)$$

where $\Delta\Delta G_{bind}$ is the difference in the free energy of binding to both receptors in the rigid body approximation as given by eq 2 and $\Delta\Delta G_{a\rightarrow h}$ is the reorganization energy difference for the transition of the RXR and RAR receptors from their apo to holo structures. In general, nuclear receptors undergo a very large conformational change upon binding involving the reorientation of some of their helices, a process that leads to the formation of the actual receptor binding site.[8] The evaluation of the latter term requires knowledge of the unbound RXRα and RARγ structures. While the structure of apo-RXRα is known, the structure of apo-RARγ is not, precluding the evaluation of the second term in eq 3. Still, as shown below, analysis of the first term and its components in eq 3 can provide some valuable insights for the design of selective ligands.

Results and Discussion

I. Ranking Affinity Prediction for Closely Related Analogues Bound to a Retinoid Receptor. Table 1 lists the known binding constants of the ligands depicted in Figure 1 for RXRα and RARγ. As seen from this table, small differences in the chemical structure of the analogues can have a profound influence on their affinity for either of these two retinoid receptors. The ligand−receptor interaction energy will be used throughout as a predictor of the relative affinity of pairs of closely related ligands for either RXRα or RARγ. Using the time evolution of this quantity, the relative affinity ranking of the compounds listed in Table 1 has been analyzed.

a. Binding of Native Retinoids. It is known that while RXR binds 9-*cis*-retinoic acid (**1**) but not *all-trans*-retinoic acid (**2**), RAR can accommodate both with similar binding affinity,[20,21] favoring slightly its cognate ligand (Table 1).[5,6] The energy profile of interaction of both stereoisomers with RARγ has been calculated, and the results are shown in Figure 2. Perusal of this picture shows that the time evolution of the interaction energy for these agonists is similar for both MD trajectories. Nevertheless, closer scrutiny of Figure 2 indicates that the lowest energy conformation is found for the RAR−**2** interaction energy profile, in agreement with the experimental results shown in Table 1, although other binding measurements indicate that both affinities are virtually identical.[10]

b. "α-Methyl" Effect. One of the features that distinguish the two retinoid receptor families is their binding site shape: whereas the binding site of RAR has an elongated, "I"-shaped form, RXR displays an "L"-shaped binding site. Hence, bent native retinoids (9-*cis*-retinoic acid; see Figure 1) are better suited than linear analogues (*all-trans*-retinoic acid) for binding to RXR.[8d] Synthetic analogues that attain an L-shaped conformation by having a bend between the hydrophobic moiety

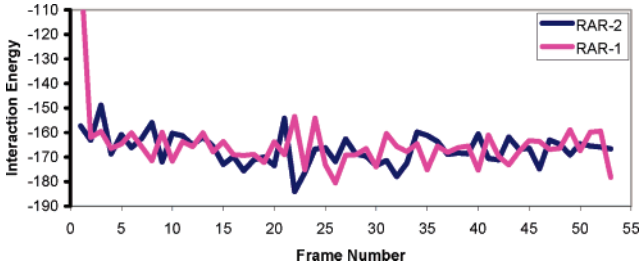

Figure 2. Time evolution of the energy of interaction between 9-*cis*-retinoic acid (**1**) as well as *all-trans*-retinoic acid (**2**) and RARγ. All energies in this work are in kilocalories per mole. The time is measured as the frame number along the MD trajectory. The time spacing between frames is 2 ps. The whole MD trajectory was used to allow us to follow the initial accommodation of the ligand in the binding site, depicted by the sudden drop in interaction energy.

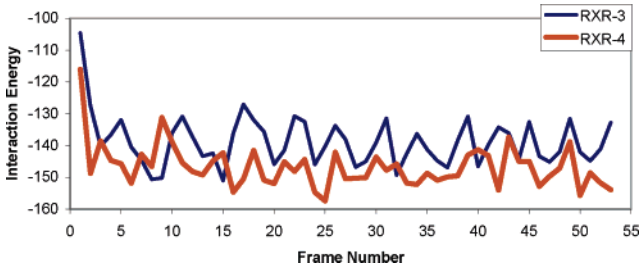

Figure 3. Time evolution for the energy of interaction between TTNPB (**3**) as well as *C*3″-MeTTNPB (**4**) and RXR. The time evolution for the orientation with the lowest interaction energy profile is displayed.

and the anchoring group should also bind strongly to RXR. Similarly to the induction of a kinked shape in 9-*cis*-retinoic acid by the C9−C10 *cis* bond, other synthetic modifications can change the binding selectivity of RAR ligands to that for RXR. For instance, it has been shown that stilbene-based arotinoids such as TTNPB (**3**) (see Figure 1) as well as benzophenone-based retinoids, which are potent RAR-selective agonists, can be transformed into RXR-selective agonists by introducing a substituent (e.g., a methyl group) located at a position *ortho* to the double bond on the tetrahydronaphthalene ring, a modification that induces a twisted conformation of the $C_{Ar}-C_{sp2}$ bond. The resulting increase in affinity for the RXR and decrease in affinity for RAR has been dubbed the "α-methyl effect".[7,10,11]

To understand this effect, we calculated the energy of interaction of both TTNPB (**3**) and its methyl analogue (**4**) with RXR and RAR. The time profiles of this quantity are shown in Figures 3 and 4.

As seen from these figures the energy of interaction of **4** with RXRα is lower than that of **3** (Figure 3). This interaction energy profile is reversed for the binding to RARγ (Figure 4). In both cases the interaction energy ranking is in agreement with the experimental ranking binding data.[7,10]

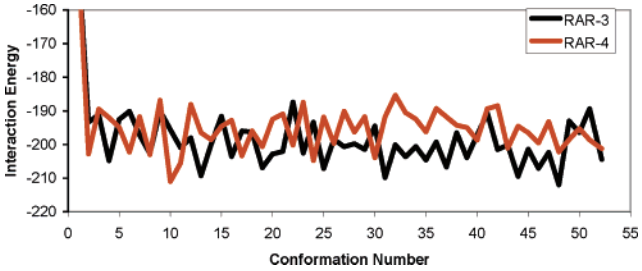

Figure 4. Time evolution of the interaction energy of TTNPB (**4**) as well as its *C*3″-methyl derivative bound to RARγ.

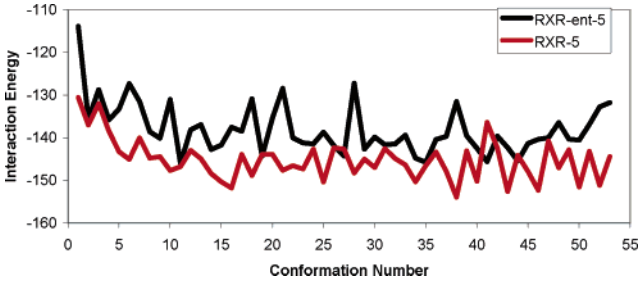

Figure 5. Time evolution of the energy of interaction of the cyclopropane-based analogues **5** and *ent*-**5** with RXRα.

c. Analogues with Cyclopropanes on the Polyene Chain. Recently, some retinoids with 9-*cis*-locked configurations induced by cyclopropane have been reported. These compounds display hypoglycemic activity in biological tests in animals.[9] The *S*,*S*-enantiomer **5** (AGN-194204) showed a higher affinity for RXRα than its antipode *ent*-**5** (AGN-194277) (see Table 1). The activity of these compounds correlates with their binding affinity, with **5** behaving as a more potent RXR agonist than *ent*-**5**.[9]

The time evolution of the interaction energy of these enantiomers with RXRα is shown in Figure 5. As seen from this figure the interaction energy of AGN-194204 (**5**) is lower than that of its enantiomer, in agreement with experimental results. The difference in affinity of these two enantiomers for RXR can be understood in terms of the van der Waals and electrostatic components of the interaction energies, as given by the first two terms and the last term of eq 1, respectively. The hydrophobic segment of retinoids and rexinoids is usually the bulkiest part of these molecules, and hence, it will make the largest contribution to the van der Waals energies, while the electrostatic term will originate mainly in the ligand's anchoring group, given its highly polar nature. The time evolution of the Coulombic interaction energy shown in Figure 6 indicates that *ent*-**5** is a poorer agonist than **5**, partly because of a less efficient interaction of its carboxylate group with the binding site polar residues. Overall, the results demonstrate the better fit of the *S*,*S*-enantiomer to the active site of RXR.

To summarize, Figures 2−5 demonstrate that the ligand−receptor interaction energy profile for pairs of structurally related analogues is a good gauge for their ligand affinity ranking for RARγ and RXRα.

II. Receptor Selectivity. a. Interplay between the Interaction Energy and Desolvation Contributions. To understand the discriminating forces that act in the binding to these two receptors, the energy contributions to the affinity of 9-*cis*-retinoic acid (**1**) for

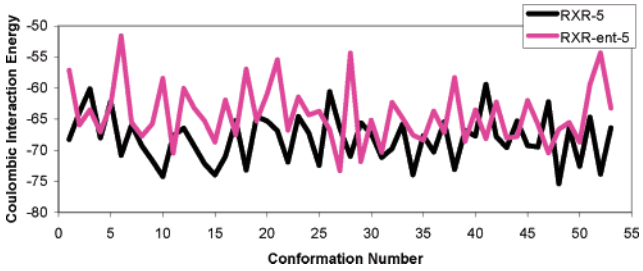

Figure 6. Time evolution of the Coulombic component of the energy of interaction of **5** and *ent*-**5** with RXRα. Notice the correlation between the interaction energy and its Coulombic contribution.

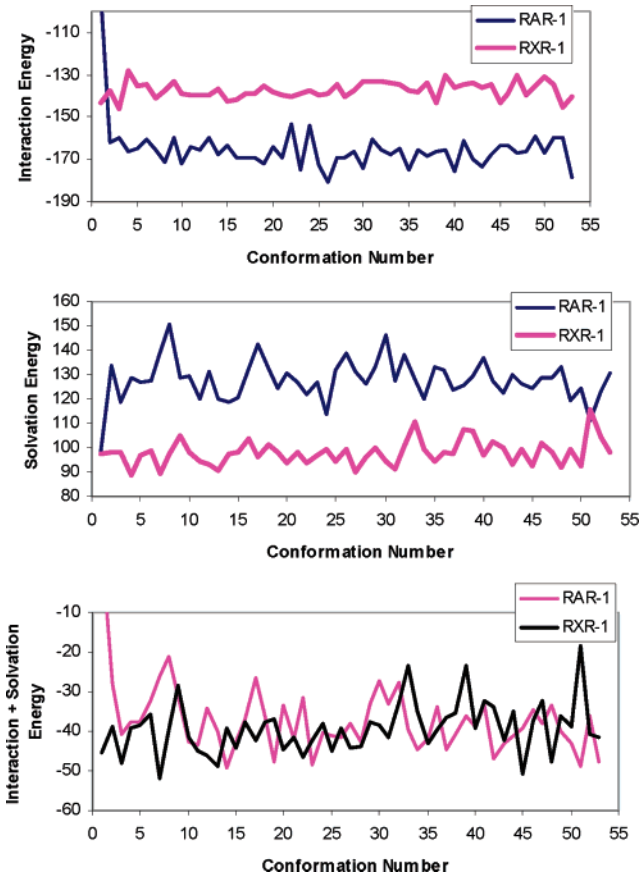

Figure 7. Analysis of the energy contributions to binding of **1** to RAR and RXR. The upper, middle, and lower panels display the time evolution of the interaction energy, the solvation energy, and the sum of both terms, respectively.

both RXR and RAR was studied. Ligand **1** binds both receptors very strongly with affinities in the nanomolar range, although binding to RAR is favored by 0.4 kcal/mol.[5,6] This difference in association energy was originally rationalized in terms of the greater number of contacts observed in the X-ray structure of the RAR−**1** complex.[8d] Analysis of the ligand−receptor interaction energy and the binding desolvation energy penalty however leads to a more elaborate explanation.

As seen from their time evolution (see Figure 7, upper panel), the interaction energy of RAR with **1** is much lower than that of RXR with the same ligand, with differences reaching 40 kcal/mol. Although the ranking predicted by the interaction energy agrees with the experimentally determined one (favoring the binding of **1** to RAR), the difference in the interaction energies for

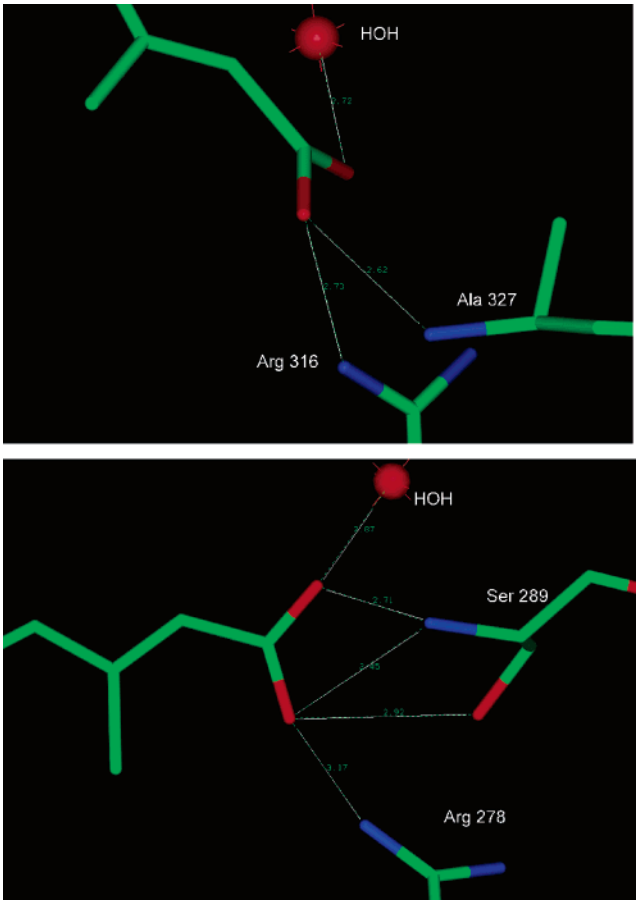

Figure 8. Close-up of the binding site region around the carboxylate group for the RXR−**1** (upper panel) and RAR−**1** complexes (lower panel), obtained from the X-ray crystallographic structures.

the binding to both receptors is much larger than the experimentally observed one. Comparison of Figure 3 with Figure 4 shows that the aforementioned interaction energy gap in the binding to RXR and RAR is also present for other ligands, such as TTNPB (**3**) and its *C*3″-methyl derivative (**4**). Perusal of the carboxylate binding region in the X-ray structures of **1** bound to RXR and RAR (see Figure 8) helps explain these large differences. Whereas the carboxylate group of **1** forms an ion pair with Arg316 and *two hydrogen bonds* (with the amide group of Ala 327 and a water molecule of RXR), it forms an ion pair (with Arg 278), as well as *three hydrogen bonds* with RAR (with the main chain amide group and the side chain hydroxyl groups of Ser 289 and a water molecule) (see Figure 8). Hence, the more negative interaction energies displayed by the RAR−**1** complex (see Figure 7) can be traced back to the additional hydrogen bond interaction between the side chain of Ser 289 and the ligand's carboxylate that is only present in the RAR−ligand complex. The binding picture would not be complete if we did not take into account the desolvation energy contribution brought about by binding. Therefore, the time evolution of the desolvation penalty contribution upon binding for both complexes was calculated. The results are depicted in the middle panel of Figure 7, which clearly shows a larger desolvation penalty for the formation of the RAR−**1** complex relative to the RXR−**1** complex, due to the sequestering of Ser 289 upon binding. The solvation contributions to the binding free energy will counterweight the difference in interaction energy, bringing the predicted free energy of binding to a closer value for both receptors, as is seen in the last panel of Figure 7.

b. Effect of Ser289 → Ala Mutations on Binding Affinity for RARγ. The interaction energy−desolvation energy compensation in the anchoring section of the binding site is borne out by findings on the effect of site-directed mutagenesis experiments upon ligand binding to RARγ.[22] Specifically, the binding affinities of **1**, **2**, and other analogues (AHPN, AGN193109, and TTNPB (**3**)) for the wild-type RARγ receptor and the mutant strain S289A were determined. The results indicate that the replacement of the polar residue Ser289 by Ala has a relatively modest effect on the ligand affinity for this receptor.[22] For most ligands the binding free energy is lowered by 0.1−0.4 kcal/mol, while for others the binding free energy increases by up to 0.7 kcal/mol when Ala replaces Ser289. These mutagenesis experiments clearly indicate that the desolvation penalty offsets almost completely and in some cases outweighs the contribution provided by the interaction energy, in support of the results shown above (see Figure 7). The actual contribution of the interaction between polar groups to the ligand−receptor binding free energy is provided to a large extent by the interaction energy−desolvation penalty compensation described above.[23−25] In some protein−ligand systems the electrostatic interaction component contributes only indirectly to the free energy of binding by selecting the optimal conformation and orientation of the ligand in the binding site,[23,24] since the compensation is almost complete. For instance, we have shown previously that the prediction of the binding free energy of some peptidic inhibitors to HIV-1 protease does not require a term depicting explicitly the polar interactions, in support of the idea that this type of contact contributes only indirectly to the free energy of binding.[24] On the other hand, we have shown that the binding ranking of some cyclic urea inhibitors with polar groups in their periphery to the HIV-1 protease can be reproduced only with a specific term that depicts polar interactions,[25] demonstrating (in that case) that the interactions between polar groups contribute directly to the free energy of binding. Some of the variables that influence more deeply the actual contribution of the polar interactions to binding are the polarity of the binding site and its exposure to solvent.

c. New Paradigms for Selective Ligand Design. The results of our work indicate that one of the main paradigms for the design of RXR- or RAR-selective ligands could be based on compounds that fully satisfy the possible interactions between the anchoring group and the polar residues lining the binding pocket in one of the retinoid receptors but not in the other. This can be achieved by designing ligands that fit better to the binding site of one of the receptors. For instance, the search of RXR-selective ligands has centered almost solely on those that have a bent shape, inducing a preferential fit to the RXR kinked binding site, as was discussed earlier in the case of **4**. Alternatively, the design of selective RXR ligands may rely on the chemical nature of the anchoring moiety. A structure could be designed that is not able to generate all hydrogen-

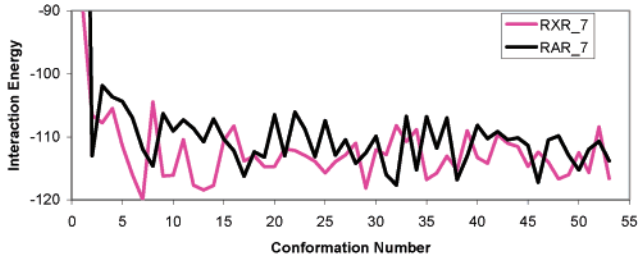

Figure 9. Time evolution of the free energies of interaction of compound **7** (in its most favorable orientation) with RAR and RXR.

bonding or ion-pair interactions when bound to RAR, while realizing all polar contacts in the binding pocket of RXR. The resulting binding energy profiles of the putative ligands to RAR would be less favorable than those of binding of analogues having a carboxylate anchoring moiety (e.g., **1** or **2**) to the same receptor. Finally, the solvation penalty contribution, which favors binding to RXR, would ensure a preferential binding of the ligand to this receptor. One of the few alternative anchoring groups used in the design of RAR and RXR agonists has been based on the 2,4-thiazolidinedione fragment. Originally, compounds with this anchoring group have been shown to be potent PPAR ligands with a clinical potential as insulin sensitizers.[26] We present here the binding analysis results for **7**, a ligand that has a 2,4-thiazolidinedione instead of a carboxylate group. This compound is very similar to MX6162 (**6**), a ligand that has been patented as an RXR-specific agonist.[14] Figure 9 displays the time evolution of the energy of interaction of **7** with RXR and RAR. The interaction energies (displayed in this figure) are closer in value for the binding to both receptors than those of the cognate retinoid ligands, favoring the binding to RXR. Inclusion of solvation energy should shift the selective binding drastically to RXR. Examination of the hydrogen bond (HB) interactions afforded by this anchoring motif provides a rationale of these results. As seen from the snapshots of some of the lowest interaction energy structures of **7** bound to RXR and RAR (see Figure 10), the anchoring group of this ligand is able to produce only two HB interactions with RAR (with the NH group of Ser289 and with Arg278), whereas the carboxylate group of RAR's cognate ligand **1** is able to produce an additional HB with the hydroxyl group of Ser289.[8]

The more favorable energy of interaction of **7** with RXR may be and probably is due to the bent shape of this ligand, which favors the positioning of the anchor in an orientation that precludes the optimal hydrogen bond contacts in the RAR binding pocket. The chemical structure of the thiazolidinedione anchoring group may also play a role by not realizing all possible polar contacts with the binding pocket residues of RAR. The actual RXR specificity could be due to a combination of the above-mentioned effects. To evaluate the weight of these contributions, we have carried out docking calculations with compound **8**, an analogue of **1** (an RAR/RXR pan-agonist), in which the carboxylate group has been replaced by a 2,4-thiazolidinedione group (see Figure 1). This compound has been synthesized, and its biological activity, based on the differentiation-inducing ability toward human promyelocytic leukaemia HL-60

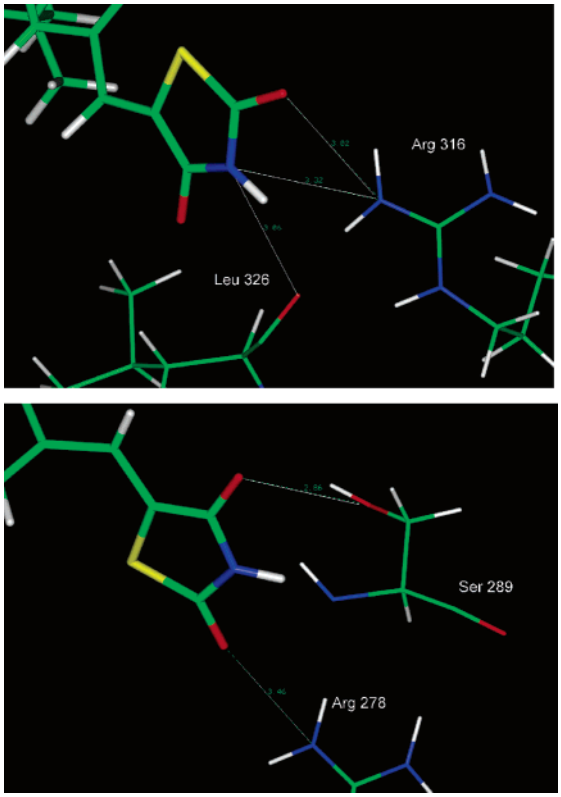

Figure 10. Snapshots of the MD trajectories for **7** bound to RXRα (upper panel) and RARγ (lower panel). These figures show close-ups of the 2,4-thiazolidinedione anchoring group and nearby residues in the binding pocket. White lines indicate atoms that are at hydrogen-bonding distances. Notice the larger number of interactions in the RXR complex.

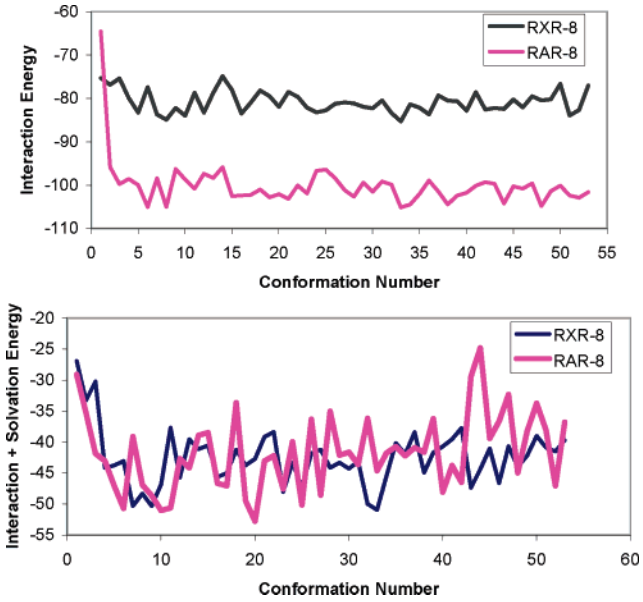

Figure 11. The upper panel displays the time evolution of the energy of interaction of **8** with RXR and RAR, while the lower panel depicts the time evolution of the free energy predictor function (see eq 2).

cells, has been determined.[27] Figure 11 displays the interaction energy profile time evolution and the binding free energy predictor as given by eq 2 for **8** when bound to RXR and RAR. The interaction energy term favors the binding of **8** to RAR over RXR, in the same way as the carboxylic acid analogues (i.e., **1** and **3**). The second

panel of this figure shows that the solvation contribution to binding cancels out most of this difference. Comparison of Figures 9 and 11 indicates that the difference in the interaction energy profile of **8** for the binding to RAR and RXR is reversed relative to that of **7**, suggesting that RXR selectivity of this latter ligand is driven to a large extent by its bent shape.

Synergistic ligand experiments in HL-60 cell lines revealed that compound **8** binds better to RAR than RXR.[27] Interestingly, the interaction energy favors binding of RAR over that of RXR (see the first panel, Figure 11). When the solvation energy is added, the differences between RAR and RXR are reduced, although the lowest energy binding conformation is still found for RAR, in agreement with experiment (see the second panel, Figure 11). Nevertheless, any final comparison between the experimental and the calculated affinities would have to wait for the calculation of the difference in reorganization energy in their transition from apo to holo structures (second term in eq 3).

Currently, we are involved in the search for novel anchoring groups that would favor intrinsically the selective binding to RXR.

To summarize, a receptor-based protocol for the docking and energy analysis of ligands to retinoid receptors is proposed and challenged with a variety of ligands that differ in the bulky hydrophobic part, the anchoring moiety, and the linker connecting them. The ranking affinity of pairs of ligands has been found to be in agreement with experiment, and the origin of their differences in RAR and RXR affinity has been rationalized. A major finding is the contrasting nature of the contribution to the free energy of binding exhibited by RAR and RXR, due to the more polar nature of the carboxylate region in the binding pocket of the former. The smaller desolvation contribution to RXR binding could in principle be exploited for the design of RXR-selective ligands that might benefit from this effect in combination with the orientation of the hydrophobic moiety with respect to the anchor group. This observation suggests that specific RXR ligands could be based on anchoring motifs that differ from the highly ubiquitous carboxylate. We have preliminarily explored this new venue with 2,4-thiazolidinedione-based analogues. This method represents a departure from the traditional design of retinoic receptor modulators based for the most part on ligand-based structure−activity relationship protocols.

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