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REGULAR ARTICLE

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New designs for inhibitors of the NF-κB: DNA binding

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Abstract We present a series of new inhibitors of the association between nuclear factor kappa B (NF-κB) and the corresponding κB site in DNA. They were designed using the lead compound 15-deoxy-$\Delta^{12,14}$ -prostaglandin J_2 (PGJ2), which is a natural product with demonstrated inhibitory efficiency for this system. First, the binding mode of PGJ2 to NF-κB was unraveled by GOLD docking calculation. Subsequently, substitutions were made to PGJ2 to optimize its association with NF-κB. Care was taken not to strongly increase the reactivity of the new compounds, and to keep the overall shape, size and hydrophilicity of the lead compound, which should render them a similar bioavailability. Molecular mechanics calculations were performed to decide on the suitability of the substitutions, and to evaluate the energies of association with NF-κB. Density functional theory calculations were performed also to study the overall reactivity of the substituted drugs towards NF-κB. Important general conclusions were obtained, concerning the improvement of these natural inhibitors; namely, a set of rational methodologies were deduced to improve the association between the PGJ2 derivatives and NF-κB, and their efficiency demonstrated by generating a set of substituted complexes, some of them with a very much increased affinity for NF-κB, opening new doors to enlarge the therapeutic capabilities of this class of drugs.

Keywords Prostaglandin · Nuclear factor kappa B · Molecular Mechanics · HIV-1 · Cancer

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Introduction

Nuclear factor-kappa B (NF-κB), is an inducible eukaryotic transcription factor of the *rel* family, and normally exists in an inactive cytoplasmic complex, with its predominant form being a heterodimer composed of p50 and p65 (Rel A) subunits, bound to inhibitory proteins of the IκB family [1–4]. The IκBs bound to dimerized NF-κB factors, block their nuclear translocation, until several physiochemical stimuli lead to proteasome dependent IκB degradation, resulting in nuclear translocation of NF-κB [4–7]. The targets of NF-κB (the κB sites) are present in the regulatory regions of the genes involved in immune (IL-1, IL-2) and inflammatory responses (IL-1, IL-6, TNF-α, TNF-β), and in genes of viruses, NF-κB/Rel members, IκB members, growth control proteins (p53, c- *myc*, Ras) and adhesion molecules [8]. Eventually NF-κB-DNA binding as a result of inappropriate activation was described to be involved in the pathology of, a number of diseases including cancer, AIDS, and inflammatory disorders [9–11]. Hence, the suppression of NF-κB was described to be of an immense pharmacological importance. In this regard several approaches, exploiting the various stages in the activation and DNA recognition of NF-κB were described [12]. These include development of several small molecules ranging from inhibitors of IκB

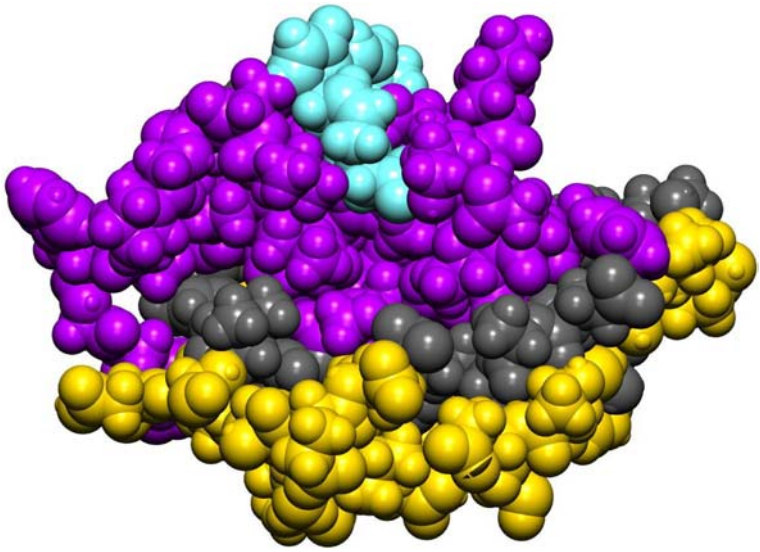


Fig. 1 The models used in this study. PGJ2 is shown in cyan bound to NF-κB model. The residues included in the 10 Å cut are shown in *purple*. The *gray* and *yellow* residues were also included in the 13 Å and 17 Å cuts, respectively

activation, and degradation to those directly binding to NF-κB [13]. One such class of potent inhibitors are the ones which covalently modify a critical cysteine residue (Cys62) in the DNA-binding loop of NF-κB-p50 homodimer. These inhibitors alkylate the Cys62 residue, and do not allow it to be in a reduced state, which is otherwise essential for major groove DNA recognition [14]. Recently, 15-deoxy-$\Delta^{12,14}$ -prostaglandin J_2 (PGJ2) was described to alkylate the Cys62 residue of NF-κB-p50 by a direct covalent modification with its electrophilic carbon [15]. PGJ2 is a biological byproduct of prostaglandin D2, which in itself is a major cyclooxygenase product [16]. It's pharmacological properties have been recently attributed to it's direct inactivation of several cellular proteins including transcription factors like NF-κB [17]. The aim of this study is to design new inhibitors based on PGJ2, binding more tightly and specifically to NF-κB-p50 protein.

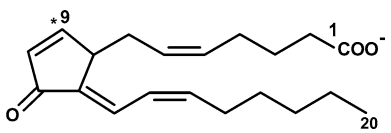
Methodology

The structure for the NF-κB p50/p50 homodimer was taken from the Protein DataBank (code 1NFK) [18], from where the DNA and one of the p50 monomers were deleted, and the other p50 monomer was used for further modeling.

The structure of the small molecule PGJ2 was designed by us. A first study was performed to find the best geometry of association between the two, with the constraint that the electrophilic carbon in the ring should be kept close to Cys62, to allow the nucleophilic addition of the thiolate of the cysteine. For that purpose GOLD 2.0 package [19] was used.

Once this first structure was obtained, the geometry of the whole system was optimized using Molecular Mechanics. However, it seemed unnecessary to include all the p50 monomer in the calculations. Moreover, the inclusion of the whole p50 monomer would imply the use of a lower theoretical level, because the calculations might become too slow. Therefore, we selected only those residues from the third of cys62, which had any atom within a radius of 10 Å and discarded all the others. This resulted in a model including the aminoacids 54–68, 107–110, 139–151 and 205–209 (1NFK numbering). To check if this smaller model was representative of the p50 monomer (in relation to the PGJ2:NF-κB binding), and no significant long range interactions were excluded in the truncation process, two additional models were built, in which the included residues were within a radius of 13 Å and 17 Å. Subsequently, the PGJ2:NF-κB binding energy was recalculated, to check if all models gave similar binding energies. The first of these two additional models (with the 13 Å radius cutoff) included all the aminoacids of the previous model plus the 53, 97, 111, 112, 138, 152–154, 201–203, 210, 237–241 amino acids and the second of these models (with the 17 Å radius cutoff) included all the aminoacids of the previous model with the 13 Å radius cutoff, plus the 50–52, 95, 96, 99–101, 105, 106, 113, 116–121, 136, 137, 155–158, 198–200, 204, 211, 212, 236, 242–247 amino acids. Figure 1 shows the three cuts of NF-κB with PGJ2 bound. The PGJ2 molecule is illustrated in Scheme 1.

All calculations were performed *in vacuum*, and the software package Gaussian98 [20]. The universal force field (UFF) for metals [21] was used to perform molecular mechanics geometry optimizations and energy calculations. We chose to



Scheme 1. The PGJ2 molecule. An *asterisk* marks the electrophilic carbon which suffers nucleophilic addition by the thiolate of Cys62. The numbering of the first, the electrophilic and the last carbon atoms is also depicted

use classical mechanics, with the UFF, to perform our geometry optimizations due to the size of the system. This is the only force field which has been parameterized for all elements of the periodic table. Several benchmarks of this force field have been published. Average errors have been determined for molecules where good quality, reliable experimental data was available. In terms of energy, typical deviations from experiment found in several organic and inorganic molecules are just a few (1–8) kcal/mol [22, 23]. These errors are somewhat systematic, and in the present case most of the errors cancel when comparing the complexation energy for the original PGJ2 and a similar substituted one. For the purpose of this work, this error amplitude is perfectly acceptable since, as will be shown, the binding energies obtained with the substitutions were much larger than these errors. The geometries are also well reproduced by this force field. In organic molecules, average deviations from experiment are 0.021 Å for C–C bond length and 0.024 Å for C–N, and 5° to 10° in angle bend [24]. The visualization of all geometries as well as all substitutions in the PGJ2 molecule was carried out with the help of programs Gaussview [25] and Molden [26].

Universal force field can use partial charges calculated by the method of charge equilibration [27]. This method generates point charges which are geometry-dependent, representing a considerable improvement in relation to traditional, non-polarizable force fields as CHARMM or AMBER [28, 29]. However, it is not an efficient procedure to recalculate the point charges at each optimization step, as this would make the calculations very slow. Therefore, the point charges were calculated only at the beginning of the optimization and at the final optimized geometry. Subsequently, it was checked whether the obtained geometry could be considered optimized using the new calculated charges. If not, a new optimization was carried out, with the point charges calculated at the end of the preceding optimization. The whole process was repeated until self consistency was obtained, i.e. the final geometry should obey the same convergence criteria with both the initial and final point charges. The overall procedure implied typically five to ten full geometry optimizations until self consistency was achieved. This approximation was tested before, by running the same calculation both determining the point charges at every optimization step, and by using the self consistent method calculating the point charges only at the beginning and at the end of each optimization. The final result was exactly the same, proving that the minimum found in both calculations is the same [30].

To calculate the energy of association we used the following procedure: we began inspecting the environment around the binding pocket of NF-κB, and introduced the appropriate substitutions in the PGJ2 molecule, (within the PGJ2:NF-κB complex); subsequently the geometry of the complex was optimized. Starting from that geometry the substituted PGJ2 was obtained by deleting the NF-κB and re-optimizing the geometry. The same was done to obtain the energy of the isolated NF-κB fragment. This same procedure was applied before to obtain the complexation energy of the unsubstituted PGJ2. The energy of association was calculated as

$$\begin{aligned}\Delta\Delta E_{\text{complex}} &= \Delta E^{\text{subs}}_{\text{complex}} - \Delta E^{\text{PGJ2}}_{\text{complex}} \\ &= (E_{\text{subs:NF}-\kappa\text{B}} - E_{\text{PGJ2:NF}-\kappa\text{B}}) - (E_{\text{subs}} - E_{\text{PGJ2}}) \\ &\quad -(E_{\text{NK}-\kappa\text{B(subs)}} - E_{\text{NK}-\kappa\text{B(PGJ2)}}) \end{aligned} \quad (1)$$

with obvious notations. Assuming that the entropic contribution for ΔG_{assoc} is similar for the original and substituted complexes, the free energy variation induced by the substitution can be approximated by the corresponding energy difference:

$$\begin{aligned}\Delta\Delta G_{\text{complex}} &= \Delta G^{\text{subs}}_{\text{complex}} - \Delta G^{\text{PGJ2}}_{\text{complex}} \\ &\approx \Delta E^{\text{subs}}_{\text{complex}} - \Delta E^{\text{PGJ2}}_{\text{complex}} \,. \end{aligned} \quad (2)$$

Results and discussion

We began by docking the PGJ2 molecule into the p50 monomer of NF-κB, with the constraint that the reactive electrophilic carbon should be close to the sulfur atom of Cys62. Specifically, a distance constraint of 2 Å was imposed between the C(9) atom of 15d-PGJ_2 molecule and the target thiolate group, and the flexible prostaglandin was allowed to freely dock around the target cysteine, in an energetically and conformationally best orientation. Docking calculations were performed in the default settings for the best possible predictive accuracy, with ten docking runs for each protein. The best scored solution (Chemscore) was considered. Chemscore gave better results due to more hydrophobic nature of the PGJ2 and inclusion of a lipophilic term in the Chemscore function [31].

The most favorable geometry was further optimized by minimization using the UFF. The result is shown in Fig. 2 below. PGJ2 binds to NF-κB through a hydrophobic pocket formed by the side chains of Tyr57, Val58, Tyr59, His141, Val142, Thr143, Lys144, Lys146, Thr150, and Leu207 (1NKF numbering). The chain corresponding to atoms C13–C20 of PGJ2 interacts through hydrophobic interactions with the side chains of Tyr59, His141, Lys144 (β and γ carbons), and Leu207. The other PGJ2 chain, comprising carbon atoms 1–8, interacts with the remaining residues. The PGJ2 molecule interacts strongly with four residues. The carboxylate at the C1 atom makes one salt bridge with the side chain of Lys146 (2.67 Å), and two hydrogen bonds with the hydroxyl groups of Thr143 (3.15 Å) and Thr150 (2.55 Å). The phenolic group of Tyr59 establishes an interaction with one hydrogen of C18 of PGJ2 (2.45 Å). The association energy was calculated in −397 kJ/mol.

Once the structure of the PGJ2:NK-κB complex is determined, new fields for rational improvement can be derived. We have followed several strategies and will discuss them one by one.

Strategy 1: Increasing the interaction between the hydroxyl group of Tyr59 and the C13–C20 chain of PGJ2

As mentioned previously, there is a strong interaction between the hydroxyl group of Tyr59 and one of the hydrogens bound

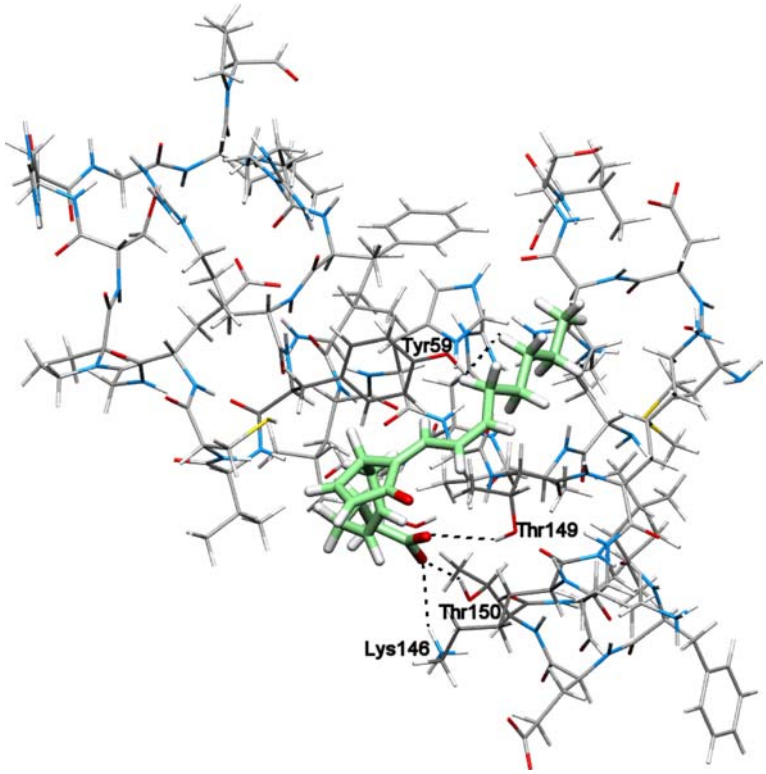


Fig. 2 The NF-κB: PGJ2 complex. PGJ2 is represented in *bold* sticks and the KF-κB cut in *thin* sticks. Relevant interactions are depicted

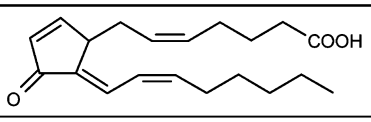
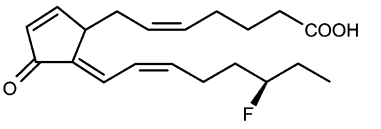
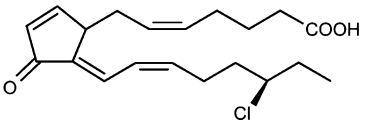
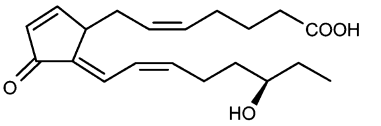
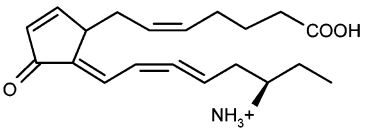
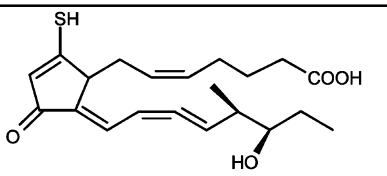
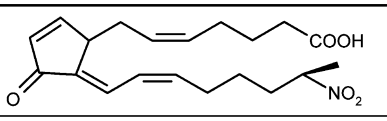
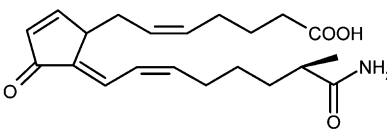
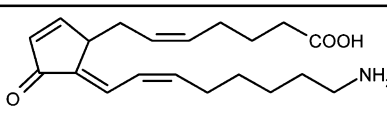
to C18. This interaction can be further increased by replacing the hydrogen on C18 by a more polar atom or group. This group can be either electronegative or electropositive, as the hydroxyl group can establish strong dipole–dipole interactions with both. Another advantage of this strategy is that it causes only a very small change in the overall size, shape and hydrophobicity of the drug, thus not disturbing its original bioavailability. Moreover, it is quite reasonable to believe that a single key atom in a single key position should increase not only affinity but also the specificity of the drug for NF-κB.

Although in the original complex the interaction is established with the hydrogen atom of C18, it is possible that substitutions in the neighboring carbon atoms (C19–C20) have a similar effect, specially if the substituents are large and therefore can still approach the hydroxyl group of Tyr59. We have tested a number of substitutions at C18, C19 and C20, which revealed favorable. The results for such substitutions are shown in Table 1. The original PGJ2 (#1) is also depicted for comparison.

As can be seen in Table 1, all substitutions at C18 (molecules number 1–6), revealed favorable (with only one exception, i.e. #6), lowering the complexation energy by 54–84 kJ/mol. It should be noticed that the calculations were performed with all ionisable groups in their physiological protonation state (e.g. the carboxylate at C1, drawn in the protonated state but treated as unprotonated, as it should be expected for a group with a pKa of 4–5 units). The increase in affinity is very significant and it is expected that these drugs are much more efficient than the original PGJ2 in the binding to NF-κB. The most promising substitution should be the one with a hydroxyl group at C18, because it is expected to be the less toxic and less reactive of the group. Compound **6** revealed a less favorable complexation energy due to the methyl group at C17, which forces the hydroxyl group to keep a larger distance from the tyrosyl hydroxyl. It was substituted also at C9 with a thiol group. That substituent is shown below to establish favorable interactions with the thiol of Cys62. Therefore, the destabilization caused by the methyl group of C17 is actually larger than suggested from the value of $\Delta\Delta G_{\text{complex}}$ (+8 kJ/mol).

Two larger polar substituents were also tested at position C19 (molecules 7–8), with the aim of establishing an interaction with the tyrosyl hydroxyl. As can be seen in the table, the results were also excellent, leading to an increase in affinity of -75 kJ/mol and -117 kJ/mol, respectively. Both the nitro and the amide substituents are not toxic and do not change the overall shape, size and hydrophobicity of the drug, and therefore are expected to represent also a strong efficiency enhancement over PGJ2 in precluding the NF-κB: DNA association. Finally, substitutions at C20 were performed with an amine substituent yielding excellent results, which show the most favorable interaction energy of all (with an $\Delta\Delta G_{\text{complex}}$ of -138 kJ/mol). This last substitution is also non-toxic and still keeps the overall bioavailability of the lead compound, thus becoming a very attractive drug to inhibit NF-κB: DNA association.

Table 1 Results (in kJ/mol) for the substitutions in carbons C18, C19 and C20. The free energy differences are expressed in kJ/mol

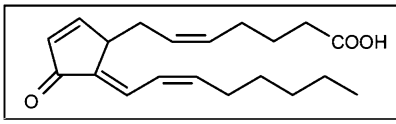
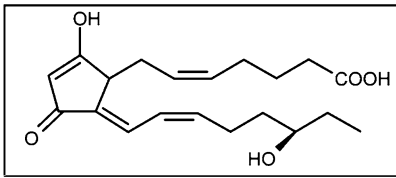
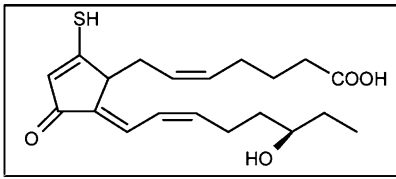
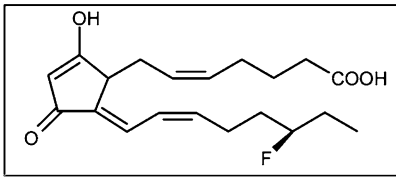
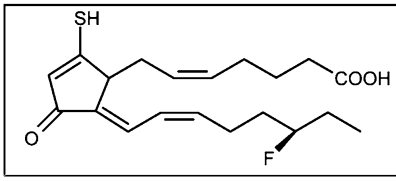
#	Molecule	$\Delta\Delta G_{complex}$
1		0
2		-84
3		-59
4		-67
5		-54
6		+8
7		-75
8		-117
9		-138

Strategy 2: Increasing both the interaction between the thiol group of Cys62 and the C8–C12 ring of PGJ2 and the interaction between the hydroxyl group of Tyr59 and the C13–C20 chain of PGJ2

Inhibition of NF-κB: DNA binding is ultimately achieved through alkylation of the thiol group of Cys62 of the p50 monomer. For this it is necessary that the PGJ2 molecule, as well as the new compounds, make complexes with an orientation that places the electrophilic carbon of the cyclopentenone ring (C9) directed to the thiol group of Cys62. As explained beforehand, most of this is achieved with the binding of the PGJ2 aliphatic chains to the hydrophobic pockets in the p50 monomer. However, substitutions that increase the interaction between the C9 atom and the thiol group of Cys62 would have the effect not only of increasing the affinity but also catalyzing the alkylation of Cys62 by generating complexes with a more productive geometry, keeping the cysteine thiol and C9 closer and more tightly attracted. To test such methodology we designed four new drugs with substitutions at the C9 position. However, to take advantage of the earlier obtained results, we tried to combine this strategy with strategy 1. Therefore, we derived a set of compounds with a double substitution, simultaneously at positions C9 and C18. The obtained results are shown in Table 2.

Several conclusions can be drawn from Table 2. To begin with, we can estimate the result of introducing a polar group at the C9 position. Comparing inhibitor **4** with **10–11** we can see that the introduction of the hydroxyl or thiol groups at C9 increases the affinity of the drugs to NF-κB. The result obtained with the hydroxyl is better than with the thiol, as expected from the larger dipole of the former. Changing the substituent in C18, from a hydroxyl to a fluorine (compounds **12–13**), leads to a further increase in affinity of the drugs to

Table 2 Results (in kJ/mol) for the double substitutions at carbons C9 and C18. The free energy differences are expressed in kJ/mol

#	Molecule	$\Delta\Delta G_{complex}$
1		0
10		-84
11		-75
12		-230
13		-209

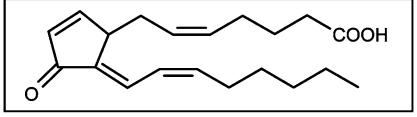
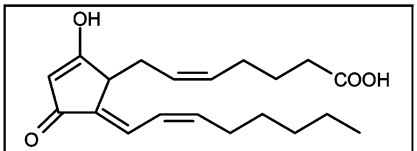
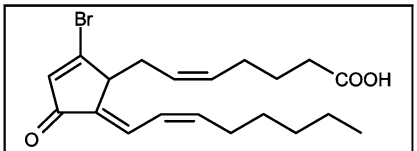
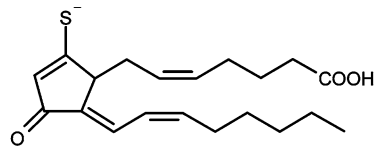
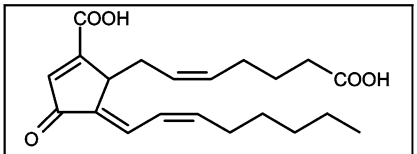
NF-κB, regardless of the substituent at C9. The same order for binding affinity is found here, i.e. a hydroxyl group at C9 is still more favorable than a thiol. The effect of introducing substituents at C9 is also very favorable here, which can be confirmed by comparing compound **2** (fluorine at C18 only) with compounds **12–13** (fluorine at C18 plus substituents at C9). The last ones show a much more favorable binding energy. In this set of new inhibitors there are also very promising drugs. Maybe the most promising (but not the one with higher affinity) is **10**, which shows a remarkable increase in binding energy (-84 kJ/mol) without introducing any substituent potentially toxic. At the other side of the spectrum we have **12–13**, which present enormous increases in affinity, but include a fluorine atom, which renders them potentially toxic, needing further tests before they would be considered as appropriate for use in vivo. We stress again that all inhibitors in Table 2 are still quite similar to the leader compound in terms of general size, and shape.

Strategy 3: Increasing the interaction between the thiol group of Cys62 and the C8–C12 PGJ2 ring – comparison of charged vs. polar substituents at the C9 position

In the preceding strategy two interaction sites were explored together. By comparing the results obtained using strategies 1 and 2, it seems that the substituents at position C9 did not have a very strong effect in affinity. It seems that the binding of the PGJ2 molecule to the NF-κB pocket hinders the mobility of the C8–C12 ring and therefore does not allow that the geometry of the complex rearranges to maximize the interaction between the substituent at C9 and the thiol of Cys62. To confirm this point and to explore ways to overcome the problem, we studied the binding of PGJ2 molecules substituted *only* at the C9 carbon. The results are shown in Table 3 below.

The results in Table 3 show that a small polar substituent as a bromine atom or a hydroxyl group have only a modest effect in increasing the affinity of the drug to NF-κB. This is mainly due to the rigid positioning of the ring relative to the thiol group of Cys62, which precludes the re-orientation of the ring in order to maximize the interaction of the substituent with the cysteine thiol. Therefore, molecules **14** and **15** have shown a $\Delta\Delta G_{\text{complex}}$ of only -4 kJ/mol. To overcome this problem, charged substituents were introduced. These establish more long-range interactions with the cysteine thiol. These interactions should still be significant even without extensive geometry reorganization. This was confirmed to be the case when a thiolate was used at C9, leading to a large increase in affinity of -192 kJ/mol. This last substitution has a limitation, which is the low abundance of the unprotonated form of the thiol group, which has typically a pKa greater than 7 by at least one unit [32]. Therefore, the most abundant form in solution will be the protonated one, resulting in a molecule similar to compound **14**, which has been shown to represent only a very modest improvement over PGJ2. Alternatively, a carboxylate substituent at

Table 3 Results (in kJ/mol) for the substitutions at carbon C9. The free energy differences are expressed in kJ/mol

#	Molecule	$\Delta\Delta G_{\text{complex}}$
1		0
14		-4
15		-4
16		-192
17		-184

position C9 is expected to be extensively unprotonated at physiological pH. The distance between the carboxylates at C1 and C9 is not large, and it should be expected an increase of the pKa for the second ionization of the molecule, due to the unfavorable interaction between both negative carboxylates. Specific interactions, as the ones that we are modeling, will overcome by a large extent the interaction between carboxylates. Assuming a pKa of four for a single carboxylate group, it would be needed a pKa shift larger than 3 pKa units to change the physiologic protonation state of the molecule, which seems to be too much in this context. However, definitive evidence would need further studies to quantify the ionization dependence between both carboxylates. This substitution has resulted in an excellent enhancement in affinity (-184 kJ/mol), the best one up until now. Again, this substituent is neither toxic nor does it change drastically the overall shape, size and hydrophobicity of the drug relatively to PGJ2, being expected to keep the overall pharmacological properties, thus becoming also a very promising drug.

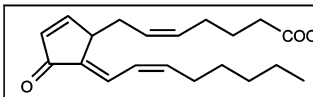
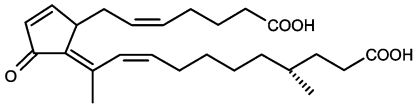
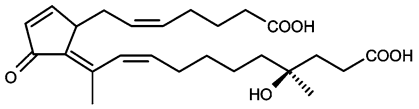
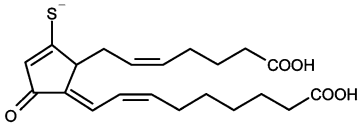
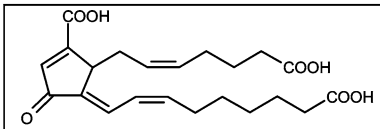
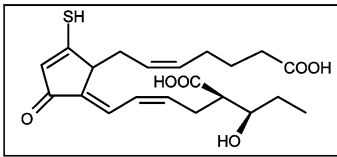
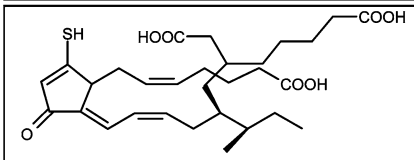
Strategy 4: Increasing the interaction between the PGJ2 molecule and residues Lys144 and Lys145 of NF-κB

There are three consecutive lysine residues near Cys62 (Lys144, Lys145 and Lys146). The last belongs to the bind-

ing pocket of PGJ2 and makes a salt bridge with the carboxylate of PGJ2. The other two (Lys144 and Lys145) are close to the binding pocket, and their side chains are not involved in any strong interaction with other residues, being instead oriented towards the solvent. Salt bridges are the most favorable interactions that exist in a proteic system. Therefore, there is the possibility of improving further the binding of PGJ2 to NF-κB by using negatively charged substituents in the PGJ2 molecule to establish salt bridges with the lysine residues. The most appropriate substituent is a carboxylate group, which shows extensive ionization at physiological pH. However, the lysine residues are still distant from PGJ2. They are both near the C13–C20 chain; the first (Lys144) has the ammonium nitrogen at a distance of 6.29 Å from C20, and the second (Lys145) has the ammonium group at a distance of 8.52 Å from C17. However, there does not seem to exist any steric hindrance that would preclude the ammonium groups to approach the C13–C20 chain. They are oriented to the solvent only because they are more stabilized by the solvent than by the aliphatic chains of PGJ2, but we can expect that they will change their orientation upon introduction of a negatively charged substituent in the C13–C20 chain of PGJ2. Another option would be to include carboxylate substituents bound to a small chain to improve their mobility. A set of new compounds was designed in order to make strong interactions with one or both the lysine residues. The obtained results are shown in Table 4.

In molecules **18** and **19** we added a propanoate substituent at position C19, which was aimed at making a salt bridge with Lys144. After performing the geometry optimization we realized that such salt bridge was indeed formed, which led to a strong increase in the affinity. In molecule **20** we also used a hydroxyl group at position C19, which was shown beforehand to make a hydrogen bond with the hydroxyl group of Tyr59. This substitution also proved successful, as the affinity was further increased over compound **18**. Compounds **20** and **21** were designed with two purposes: first, to see to which extent the C13–C20 chain needed to be increased in order to make a salt bridge with Lys144. Therefore, a carboxylate group was directly attached to C20. Second, we tried to take advantage of the earlier results with molecules **16** and **17**, and added also a charged group at position C9. Both substitutions revealed very favorable, leading to a very large increase in affinity of -241 kJ/mol, the largest of all the compounds we have designed. Molecules **22** and **23** represent an attempt to establish another salt bridge with Lys145. In compound **22** we introduced one acetate substituent at position C17. This substitution proved to be favorable (compare with molecule **11**, which is similar except in the methylcarboxylate substituent), although not as much as the previous ones. The acetate chain proved to be too short for an optimal interaction with Lys146 to occur. Therefore, we increased that chain in molecule **23**, with two branches terminated by carboxylate groups, each one oriented towards one of the two lysine residues. This molecule displayed a high affinity due to the two salt bridges which establishes with NF-κB. We did not try to add another carboxylate at C9 here (which would result in a further affinity increase) because it could eventually change too much the hydrophobicity of the molecule and consequently it's bioavailability. In summary, the largest increases in affinity (the most promising drugs) are obtained by introducing negatively charged substituents that make salt bridges with the NF-κB residues Lys144 and Lys145.

Table 4 Results (in kJ/mol) for the carboxylate substituents. The free energy differences are expressed in kJ/mol

#	Molecule	$\Delta\Delta G_{complex}$
1		0
18		-147
19		-170
20		-241
21		-241
22		-133
23		-213

Conclusions

We have studied the PGJ2: NF-κB association, and based on that we have derived a set of strategies to improve the association with only small and subtle changes in the PGJ2 molecule. As far as possible, the general size, shape and polarity of the molecule were not altered, in order to keep its good bioavailability. The most promising methodologies to improve the association were found to be the following: (1) to add polar/charged substituents at carbon C9 that make hydrogen bonds/ionic interactions with the Cys62 thiol

group (2) to add polar/charged substituents at carbon C18 to make hydrogen bonds/ionic interactions with the hydroxyl group of Tyr59 (3) to add negatively charged groups, connected to small aliphatic chains, to make salt bridges with Lys144 and Lys145. The overall result is very promising. We have obtained a set of 23 compounds which show a marked increase in affinity to NF-κB. Moreover, as these compounds were designed to complement the binding pocket of PGJ2 in NF-κB it is quite reasonable to expect that their selectivity in vivo should be much increased too. Therefore, we expect that these compounds are highly promising drugs to preclude NF-κB: DNA association and therefore gene transcription.

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