Induced fit and pharmacophore generation approach applied to A_{2A} adenosine receptor antagonists

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Abstract

Receptors A_{2A} have an important role in the regulation of mood and motor activity; the available evidence has provided the basis for the formulation of a theory according to which selective A_{2A} adenosine receptor antagonists can be useful for the treatment of Parkinson's Disease. To explore the binding properties of some adenosine-like antagonists, we have employed two different approaches: (1) induced fit applied to an apo 3D receptor model, and (2) 3D QSAR. We have applied the Glide/Prime induced fit protocol developed by Schrödinger, using the apo-receptor structure and one of its known antagonists, in order to obtain a 3D receptor-antagonist complex structure. The IF protocol led to a receptor structure able to bind adenosine-like antagonists that can be used in future docking studies. To highlight the spatial arrangement of chemical features that confers drug activity toward the A_{2A} receptor, two different pharmacophore hypotheses were generated with Phase, using 68 A_{2A} antagonists retrieved from literature. Both hypotheses suggested a binding mode consistent with previously published site-directed mutagenesis and SAR studies. The predictive power of these 3D QSAR models is still under development.

Keywords: Adenosine receptor, docking, induced-fit, pharmacophore generation, 3D QSAR

Introduction

Adenosine modulates a great variety of physiological functions, such as a general depression of the central nervous system, vasodilation and inhibition of platelet aggregation.[1] These effects are mediated by different subtypes of specific membrane receptors pharmacologically classified as A_1, A_{2A}, A_{2B} and A_3.[2] Activation of these receptors inhibits or stimulates the intracellular enzyme adenylate cyclase (AC). A_1 and A_3 receptors are coupled to the inhibitory G-protein Gi/Go, while A_{2A} and A_{2B} receptors are coupled to the stimulatory Gs protein, thus stimulating AC activity. In

recent years it has become more and more clear that adenosine receptors may be targets for the development of new drugs. Among the pathological conditions that might be treated with agonists or antagonists of adenosine receptors there are Parkinson's disease, hypoxia/ischemia, epilepsy, kidney disease, asthma and cancer.[3]

In particular, there is enough experimental evidence that selective A_{2A} adenosine receptor antagonists can be useful for the treatment of Parkinson's Disease.[1]

Although several structure-activity relationship studies (SARs) on different chemical classes of human A_{2A} antagonists have been published and the development of more potent and/or selective antagonists is still being pursued intensively, only a few studies have investigated the structural basis of molecular interaction between these antagonists and the A_{2A} receptor.

At present one 3D model of the human A_{2A} adenosine receptor built by homology modeling from rhodopsin is available (pdb code 1UPE). The model was generated with no ligand in the active site. The residues forming the active site cavity in the receptor were recognized by means of site-directed mutagenesis studies that highlighted also the important residues for the antagonists binding to the receptor.[4]

Aim of this study is to generate a 3D model of a complex of A_{2A} receptor and a known antagonist able to rationalize the binding properties of some known A_{2A} antagonists, and to set up the basis for a future structure-based design project, such as virtual screening. To achieve this objective we have employed two different approaches: (I) Induced fit protocol (Structure based design approach) and (II) 3D pharmacophore generation procedure (Ligand based design approach).

The Induced Fit Protocol developed by Schrödinger, is an innovative method for modeling the conformational changes induced by ligand binding. It uses the docking program Glide to account for ligand flexibility and the Refinement module in Prime[5] program to account for receptor flexibility.[7] Induced fit generates alternative receptor structures able to accommodate different ligand structures. It has two main applications: generation of a complex structure for a ligand known to be active but that cannot be docked in an existing (rigid) structure of the receptor, and rescue of false negative employing more than a single receptor structure for the virtual screening. We have tried to obtain an alternative 3D structure of the A_{2A} apo receptor, with the active site cavity geometry adjusted to bind the well-known antagonist CGS15943[4].

Even when, as in this case, a protein structure-based approach is possible as the structure of the target is known, a ligand-based approach may provide an alternative and complementary tool for drug design. Therefore we have generated a pharmacophore model using a set of antagonists of A_{2A} receptor retrieved from literature, in order to find out the spatial arrangement of chemical features that confers drug activity toward the selected receptor.

Results and Discussion

Model optimization using induced fit protocol

First of all, a preliminary docking study using the publicly available 3D structure of the A_{2A} receptor was carried out. Glide was employed to dock CGS15943 into the receptor structure. As expected, no ligand poses into the binding site cavity were found, obviously due to a closed conformation of the active site. The Induced Fit Protocol was so employed to generate an alternative conformation of the receptor able to bind the specific ligand.

The procedure has four steps: (1) initial softened-potential docking into a rigid receptor to generate an ensemble of poses; (2) sampling of the protein for each ligand pose generated in the first step; (3) redocking of the ligand into low energy induced-fit structures from the previous step; and (4) scoring by accounting for the docking energy (GlideScore), and receptor strain and solvation terms (Prime energy). Accuracy is ensured by Glide's superior scoring function and Prime's advanced conformational refinement[7].

The challenge in the first step is to generate at least one reasonably docked pose for the ligand (independent of the score it receives). Without a plausible initial guess for the ligand pose, any attempt to predict reorganization of the protein structure is unlikely to succeed in the context of a limited allotment of CPU time. The principal challenge in the second step is predicting the low energy receptor conformation for a correct ligand pose, starting from the "plausible" initial guess generated in the first step. The primary challenge in the third step is to generate low energy ligand conformations when presented with the correct receptor conformation. The difficulty in the final scoring step lies in properly ranking the complexes such that the top ranked pose correctly predicts the ligand/receptor structure.

The analysis of the generated models was made using as guideline a published assumption of binding mode for antagonists, well consistent with the experimental results and the evidence emerged from mutagenesis studies[4] (Figure 1). In particular, we have focused our attention to the occurrence of the hydrogen bond interaction between the ligand and the residue N253, which seems necessary for antagonist's activity; additional H-bonding between side chain of N181 and N^6 of the CGS15943 is not necessary but could increase the stability of the complex, so the position of the side chain of this residue was also taken in account during the analysis of the generated models.

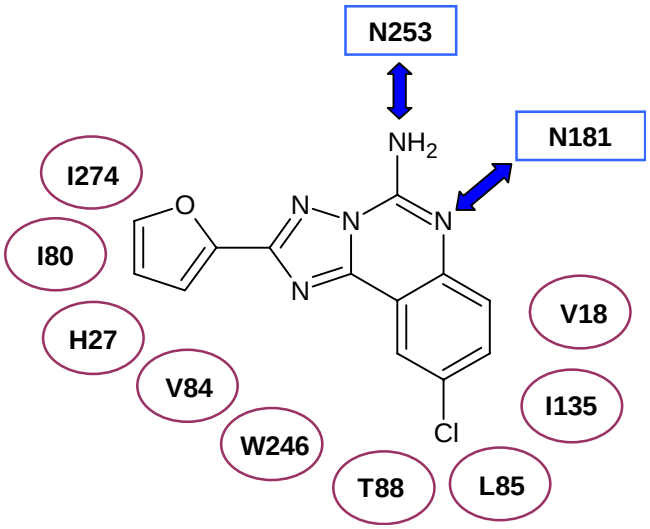


Figure 1. Binding mode of CGS15943 in the A_{2A} active site cavity; residues involved in hydrophilic interaction are identified by a rectangle, while an ellipse identifies residues involved in hydrophobic interaction.

The selected model is shown in **Figure 2**. When compared with the original model, the IF model shows an enlargement of the active site cavity mediated by side-chain flipping of the residues F182, W246, H250, N253, N181 that allow a fit of CGS15943 perfectly consistent with the binding hypothesis previously reported. The quality of this model in comparison with the original one, was subsequently validated using the Ramachandran plot, generated using the software RAMPAGE[8]. The fraction of the residues falling in outlier region was lower than that of the published model, and all the residues involved in the binding of the antagonist are in favoured region.

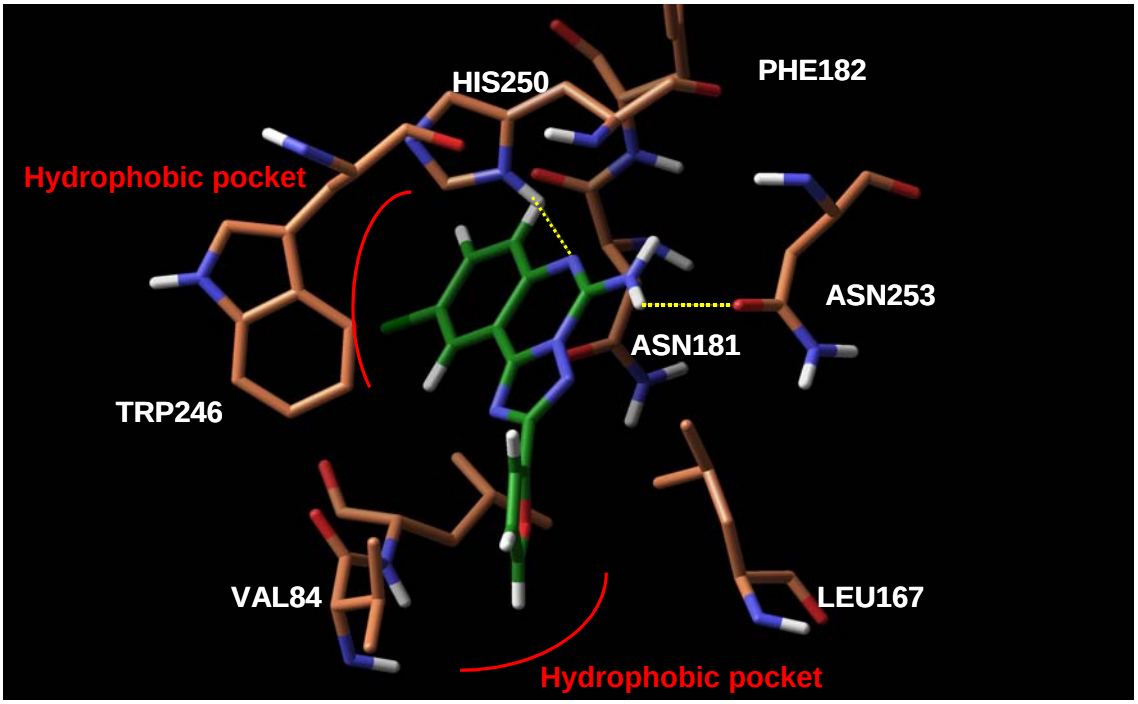


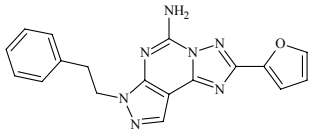
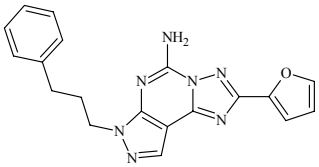
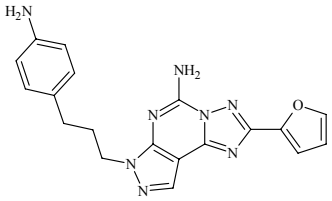
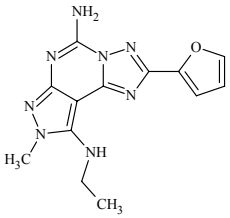
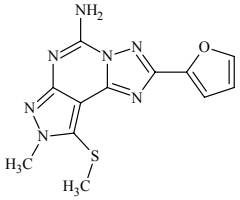
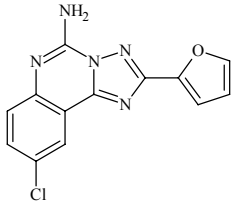
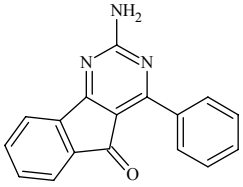
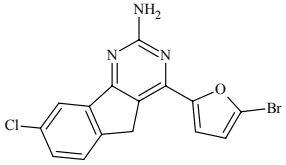
Figure 2. Active site of induced fit model.

Pharmacophore model generation

The preliminary data set for pharmacophore generation included 320 antagonists retrieved from literature and respective inhibition constants (Ki).[9-20] We selected the training set using chosen molecules with human binding data, defined stereochemistry and biological data (e.g. structure with biological data such as Ki>500 were excluded from the training set). The final training set was composed by 68 molecules (data not shown). 8 molecules were then chosen with high activity, Ki< 10 nM, and structural diversity as the "pharm set" that will be used for model development (Table 1).

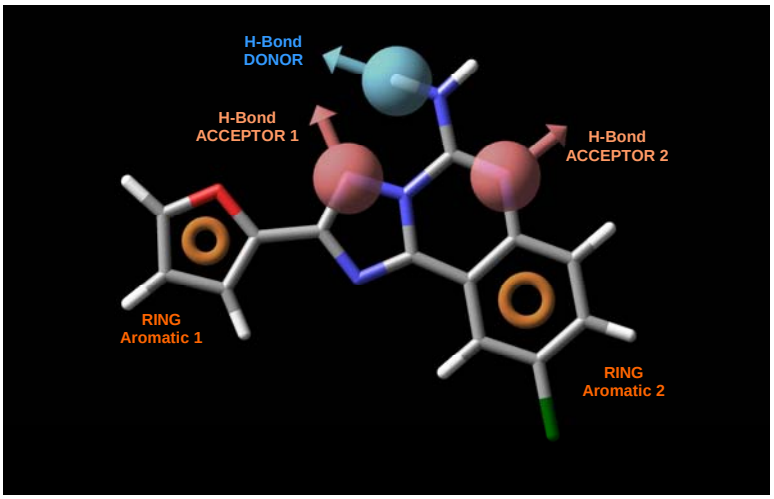
Two different pharmacophore hypotheses were generated using the software Phase[6], both suggesting a binding mode similar to our original working hypothesis. The pharmacophore 1 contains two hydrogen-bond acceptor features, two aromatic ring features and one hydrogen-bond donor feature, while the pharmacophore 2 contains two hydrogen-bond acceptor features, one hydrogen-bond donor feature, one aromatic ring and one hydrophobic feature. Figure 3 shows the pharmacophore 1 mapped onto CGS15943 (**6** in table 1) and pharmacophore 2 mapped onto **5**.

Table 1. Molecules included in the pharm set and respective activity data

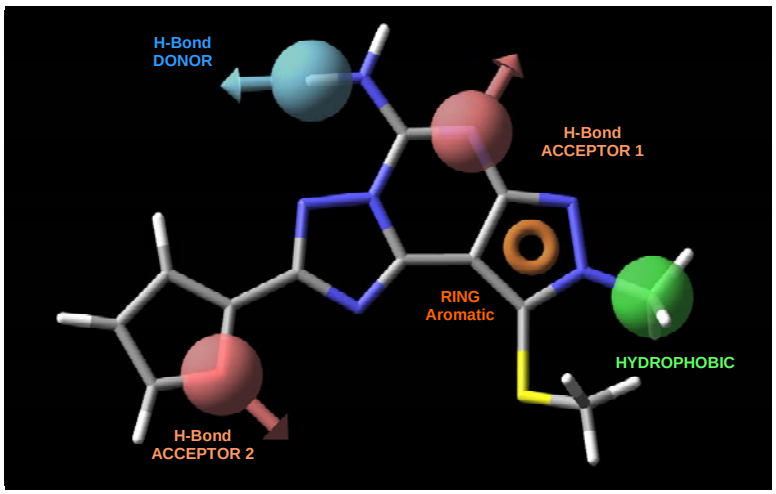
N°	Structure	A_{2A} Activity Ki (nM)	N°	Structure	A_{2A} Activity Ki (nM)
1		1.1	**2**		1.2
3		0.22	**4**		10
5		1.2	**6**		1.2
7		1.7	**8**		1

It is interesting to note that all key interactions between strong antagonists and the A_{2A} receptor, as reported in figure 1, are encoded in both pharmacophore hypotheses. In particular: the H-bond between the exocyclic amino group at the 5-position and N253, the H-bond between the side chain of N181 and the endocyclic nitrogen at 6-position, the two large hydrophobic interactions near the quinazoline ring and in proximity to the furan ring are revealed by the pharmacophore 1, whereas the hydrophobic interaction near the furan ring is replaced by an H-bond acceptor feature in the pharmacophore 2.

At the moment, there is no clear evidence about the role of the furan ring in the binding of antagonists, and this appears as an interesting challenge for future works. Some SARs studies[10] suggest that the furanyl ring at the 2-position of the tricyclic structure is a necessary element to guarantee the activity of the antagonists, probably because of the oxygen atom that may produce a favorable electronic interaction with the adenosine receptor. On the other hand, the 3D structure of the antagonist-receptor complex generated via induced fit does not highlight any kind of hydrophilic interaction in the binding site region occupied by this moiety.



Pharmacophore 1



Pharmacophore 2

Figure 3. Pharmacophore models generated by Phase.

Conclusions

The results presented above demonstrate that the IF protocol could generate 3D structures of a receptor of good quality to be used for subsequent structure-based drug design efforts. In this particular case, the use of both a ligand-based approach and a structure-based approach, could lead to a more complete knowledge of the mechanism of binding.

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