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ORIGINAL PAPER

Homology modeling and flex-ligand docking studies on the guinea pig β_2 adrenoceptor: structural and experimental similarities/ differences with the human β_2

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Abstract The trachea of a guinea pig is widely used in drug development assays focused on the treatment of pulmonary diseases. Some of these drugs relax the airways by binding to the guinea pig β_2-adrenoceptor (Gβ_2AR). In this work, the amino acid sequence of the Gβ_2AR was searched to carry out homology modeling, using the Swiss-Model server, with the human β_2AR as the parent template. The Gβ_2AR 3-D structure was structurally and energetically optimized *in vacuo* using NAMD 2.6 program. The refined 3-D model obtained was used for further study. Molecular docking simulations were performed by testing a set of well-known β_2AR ligands using the AutoDock 3.0.5 program. The results show that the homology model of Gβ_2AR has a 3-D structure very similar to the crystal structure of recently studied human β_2AR. This was also corroborated by identity (94.23%), Ramachandran map, and docking results. The theoretical simulation showed that the ligands bind at sites that are similar to those reported for the human β_2AR. The R-enantiomer ligands showed correlation with in vitro data. We have obtained a Gβ_2AR 3-D model which can be used to carry out computational screening as a complementary tool during the drug design and experimental tests under guinea pig models.

Keywords β_2 adrenergic receptor ligands · Docking · Drug development · Guinea pig · Homology modeling

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Introduction

The β_2 adrenergic receptor (β_2AR) is a target receptor used for drugs designed to treat asthma, among others respiratory diseases [1]. It is also a good model for studying the behavior of G protein coupled receptors (GPCR) [2]. Experimental models for testing the effects of ligands on β_2AR have been widely employed in drug development. Guinea pigs have been the most commonly used small animal species in preclinical studies related to asthma and chronic obstructive pulmonary disease [3] because the potencies and efficacies of agonists and antagonists on their airways are similar to the effects on human airways. Guinea pigs and humans exhibit many similarities in physiological processes, especially airway autonomic control and the response to allergens [4]. Their β_2AR structural differences are minimal, and can be attributed to the constitutive amino acids or regulatory activity associated with the different polymorphisms in each species [5]. These structural differences are possible reasons for the discrepancies in the results from preclinical studies. However, the amino acids between species at the binding site are conserved [4, 5]. Thus, guinea pig models have played an essential role and offer multiple advantages for studying physiological or physiopathological processes. One of these advantages is the possibility for studying β_2AR stimulation by agonists or receptor blocking by antagonists [4, 6–8]. These models have been useful in drug design and for

experimental research that tests a variety of drugs without having to use human subjects [3].

Recent experimental procedures (X-ray) have provided 3-D models [9–11] of the human β_2AR (Hβ_2AR). These models have been used for gaining insight into GPCR ligand recognition and activation [12–14]. The results from these models are in agreement with in vitro assays on Hβ_2AR [11, 14]. Currently a 3-D model does not exist for the guinea pig β_2AR (Gβ_2AR). The theoretical insight from this model is a necessity for evaluating the drug β_2AR action on guinea pig models and for comparison with the in vitro studies.

In this work, a guinea pig β_2AR 3-D model (Gβ_2AR) was built. Well-known ligands were docked in order to demonstrate the capability of obtaining specific interactions between the ligand and the receptor. We identify the relationship with previous experimental reports, the potential for these models as a complementary tool for ligand behavior evaluation in vitro drug development tests in guinea pig models.

Methods

Primary sequence analysis and sequence alignment

The NCBI protein data base [http://www.ncbi.nlm.nih.gov/sites/entrez] was used to search the sequence of amino acids for Gβ_2AR. The complete sequence was reported by Oostendorp (ID:Q8K4Z4, entry name ADRB2_CAVPO, 418 amino acids) [5]. BLAST server was used to carry out the homology modeling by aligning Gβ_2AR sequence with the β_2AR template protein sequences.

Tertiary structure prediction of Gβ_2AR and validation

Gβ_2AR 3-D homology models were built based on the 3-D structure of Hβ_2AR (PDB codes: 2r4r, 2r4s and 2rh1) using the Swiss-Model server [15, 16]. The 3-D models were subsequently analyzed and selected according to the best fit of the sequence identity. The best identity (94.2%) was the Hβ_2AR model with PDB code: 2r4r, which was used for subsequent steps. Then, hydrogen atoms were added to the Gβ_2AR model, which was at pH~7. The structure was then minimized using steepest descendent (SD) protocol, with 10000 steps, using NAMD (v2.6) [17]. A Ramachandran plot was generated by Visual Molecular Dynamics (VMD1.8.6) [18]. This plot shows the allowed and disallowed regions for the modeled molecule. Additionally, a quantitative assessment of the quality of the protein structure predictions, relative to its parent structure, was done by comparing Gβ_2AR and its template (2r4r) using the TM-score program (an algorithm that calculates the topological similarity between two protein structures) [19].

Retrieval of ligands

A set of ligands, which includes well-known β_2AR agonists and antagonist (Schemes 1 and 2, respectively), was use to determine the binding energy and binding modes between ligand and the Gβ_2AR. Their 2-D structures were downloaded from the DrugBank [http://www.drugbank.ca/drugs] and were used to confirm the chemical structure. R enantiomers are known as agonists, whereas S enantiomer forms for inverse agonist and antagonist have been reported experimentally as eutomers [11, 20]. Both the R and S enantiomers for each compound were modeled and used for further study. The minimum-energy 3-D structure for each ligand was obtained by Gaussian 98 software using the B3LYP/6–31G* level [21].

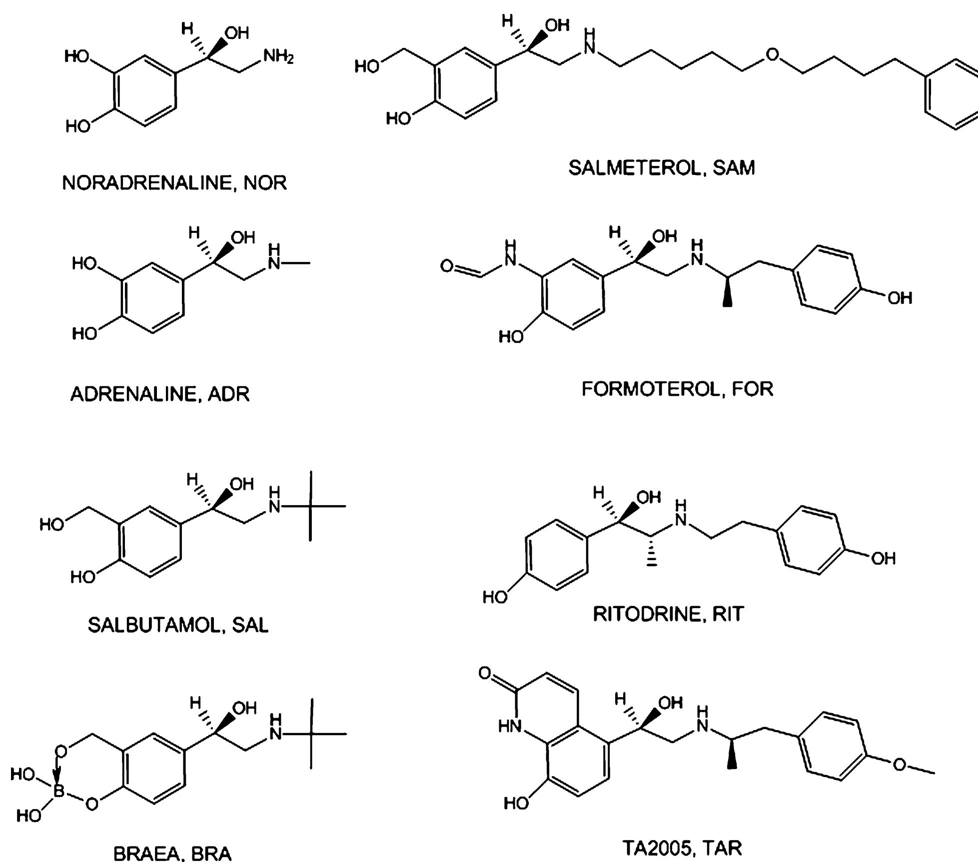
Docking simulations and determination of binding affinity

Kollman charges for all Gβ_2AR atoms, solvent parameters and non-merge hydrogen were assigned by using AutoDock tools 1.5.2 [22]. In order to identify the ligand recognition on the Gβ_2AR binding sites, the possible rotatable bonds, the torsions and the atomic partial charges (Gasteiger) of the ligands were assigned by using AutoDock tool 1.5.2 [22].

Next, the ligands were docked inside a cubic grid box ($80\times80\times80$ Å) centered at the D113 α carbon (D113 is always conserved at the β_2AR putative binding site). The grid points separated by 0.375 Å on the Gβ_2AR by using the AutoDock 3.0.5 software. This docking simulation was achieved under the hybrid Lamarckian genetic algorithm, which had an initial population of 100 randomly placed individuals and the maximum number of energy evaluations set at 25×10^7. The resulting docked orientations within a root-mean square deviation (RMSD) of 0.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound was used for further affinity and conformational binding analysis. All other parameters were maintained at their default settings [22]. Visualizations were performed by using VMD 1.8.6 program [18].

Comparison between *in silico* simulations and in vitro assays on Gβ_2AR or Hβ_2AR

Affinity values from well-known ligands (pD_2 or pA_2) were obtained from in vitro models and compiled [23–31]. After docking simulations, the AutoDock tool 1.5.2 was used to obtain the intermolecular affinity values (free energy and pK_D) for the five ligand-Gβ_2AR complexes with lowest free-energy (highest affinity) [22]. Next, the mean and standard error for these values were calculated. These results were compared with affinity values reported from in vitro assays on guinea pig trachea [24–31]. For both, theoretical and experimental data, the coefficient of determination was calculated. Additionally, this data was compared with data



Scheme 1 Partial and full R-agonists tested on the Gβ$_2$AR model. Only the common chiral center for all ligands was considered for building R/S 3-D structures

obtained with a similar theoretical methodology for Hβ$_2$AR (presented by our workgroup in the 33rd FEBS congress and 11th IUBMB conference 2008. Athens, Greece).

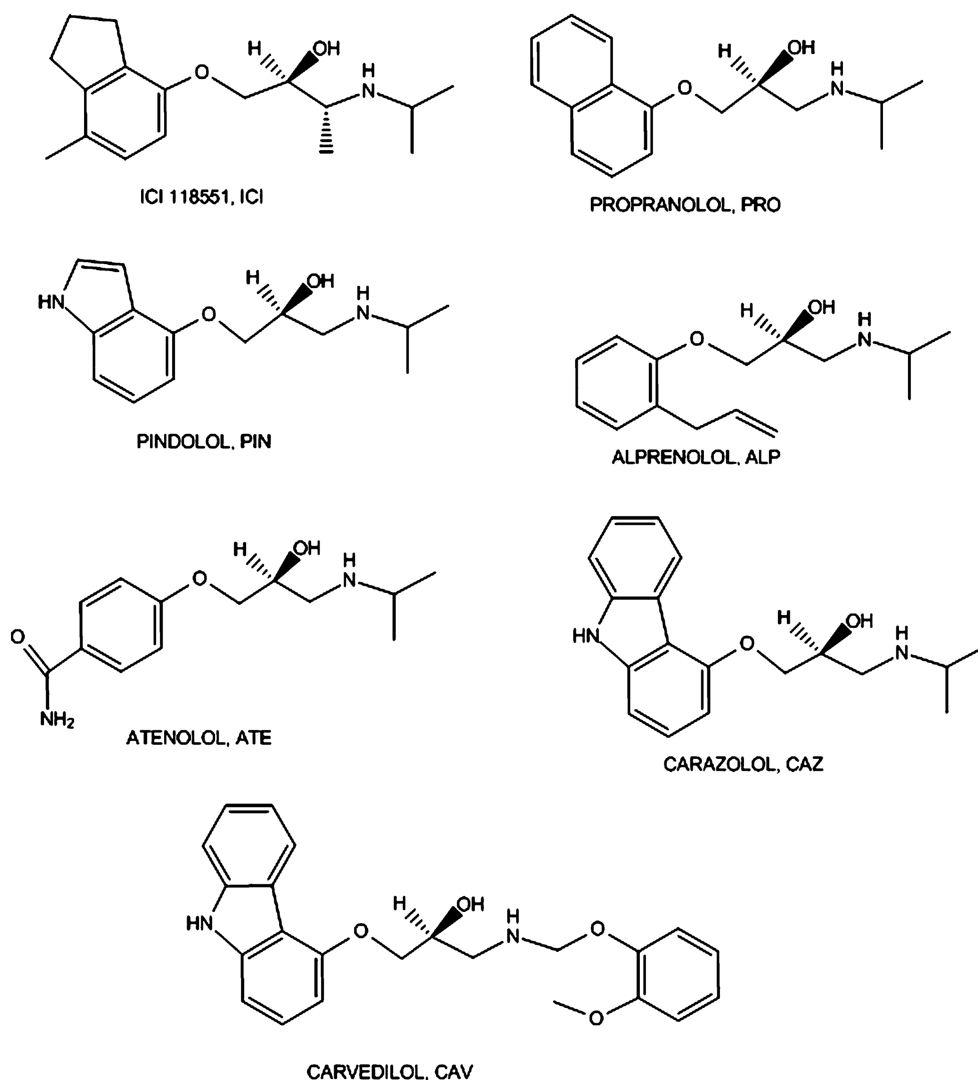
Results and discussion

Homology modeling

The guinea pig models (3) obtained had at least 92.2% of identity according to homology model obtained from a Swiss-Model server with recently reported Hβ$_2$AR structures (Table 1) [9, 10]. This receptor was successfully built, in accord with the fact that receptors with homology greater than 30% can be used as templates adequately [32], together with a homology that has shown a RMSD 1 Å greater than 50% of 3-D structures. Initially, the model obtained, using the PDB: 2rh1 model as the parent structure, contained only five transmembranal domains (TM). Because this sequence included the lysozyme bonded in chain A (Fig. 1). Whereas the model obtained from 2r4r parent template contained seven TM like all classical GPCR described [9]. Therefore, this parent model was selected and employed in our Gβ$_2$AR 3-D homology study.

The structure of the Gβ$_2$AR model obtained was verified through a Ramachandran plot (Fig. 2). This plot showed the allowed linkage torsion angles, which could be consistent with the experimental results for Gβ$_2$AR. The Gβ$_2$AR model selected (seven TM) was compared with its template by superimposing the 3-D shapes. It was then energetically optimized using NAMD v2.6 [18]. Its TM-score was 0.8764. Therefore the model does not correspond to a random selection from the PDB library [19].

Some of the Hβ$_2$AR residues reported play an essential role in the ligand binding event [9–11, 14]. Figure 3 shows those that are exclusive for the guinea pig receptor, both the parent and resulting models are superimposed. The binding pocket for both 3-D models, Hβ$_2$AR and Gβ$_2$AR, showed similar dimensions and spatial distribution (Fig. 3). However, the residues in Hβ$_2$AR form a slightly stretched cavity, which does not appear important in affecting the affinity for a majority of the ligands. That is judged by pK$_D$ calculated values on Hβ$_2$AR and Gβ$_2$AR 3-D models (Chart 1). The comparison between *in silico* (for R-enantiomer structures) and in vitro data showed that all, compiled and calculated data, follow same tendency (Chart 1). Those compounds with greater affinity reported for in vitro assays also showed greater affinity for *in silico* experiments. Some exceptions were identified for the Hβ$_2$AR model. This could be attributed to the bulky moieties bonded to the amino group (Scheme 1, Chart 1) of these ligands (Salmeterol, SAM and Formoterol, FOR). Moreover, the high hindrance effects that



Scheme 2 Antagonists and inverse agonists tested on the Gβ_2AR model. The common chiral center for all ligands was considered for building R/S 3-D structures

allow the ligands to interact outside of the H/Gβ_2AR binding site, which could be different between the two receptors tested. We have identified that the Gβ_2AR built has different amino acids than Hβ_2AR (PDB code: 2r4r). These amino acids are not in or near the binding site region, however, they can do interfere with the ligand affinity. This can explain why the biological model of the guinea pig airway can be applied for researching drugs that treat human airway diseases such as asthma [3, 24]. However, it is possible that the different amino acids can provoke a distinct conformational state in the binding pocket in a dynamic state. Their position can modify the inter-residues interaction or with lipid bi-layer membrane and cholesterol molecules recently implicated in the β_2AR action (see Fig. 3) [11]. Although they cannot modify the ligand recognition. The residues, with polymorphisms responsible for the expression regulation receptor in human (amino acids 16 and 27) [26], and guinea pigs [5], were not visible. They are present at the

Table 1 Models obtained by homology model with Hβ_2AR models as a template structure

Model used as template (PDB code).	Sequence identity (%) [a]	Modeled residue range	Notes
2rh1	92.20	29 to 230	The built model is confirmed by five TM's, the end-point coincide with the binding point for T4-lysozyme at the template.
2r4r (**Gβ_2AR**)	94.23	37 to 348	The built models have seven transmembranal domains.
2r4s	94.21	37 to 348	

[a] Reported by Swiss-model server automated system [16, 17].

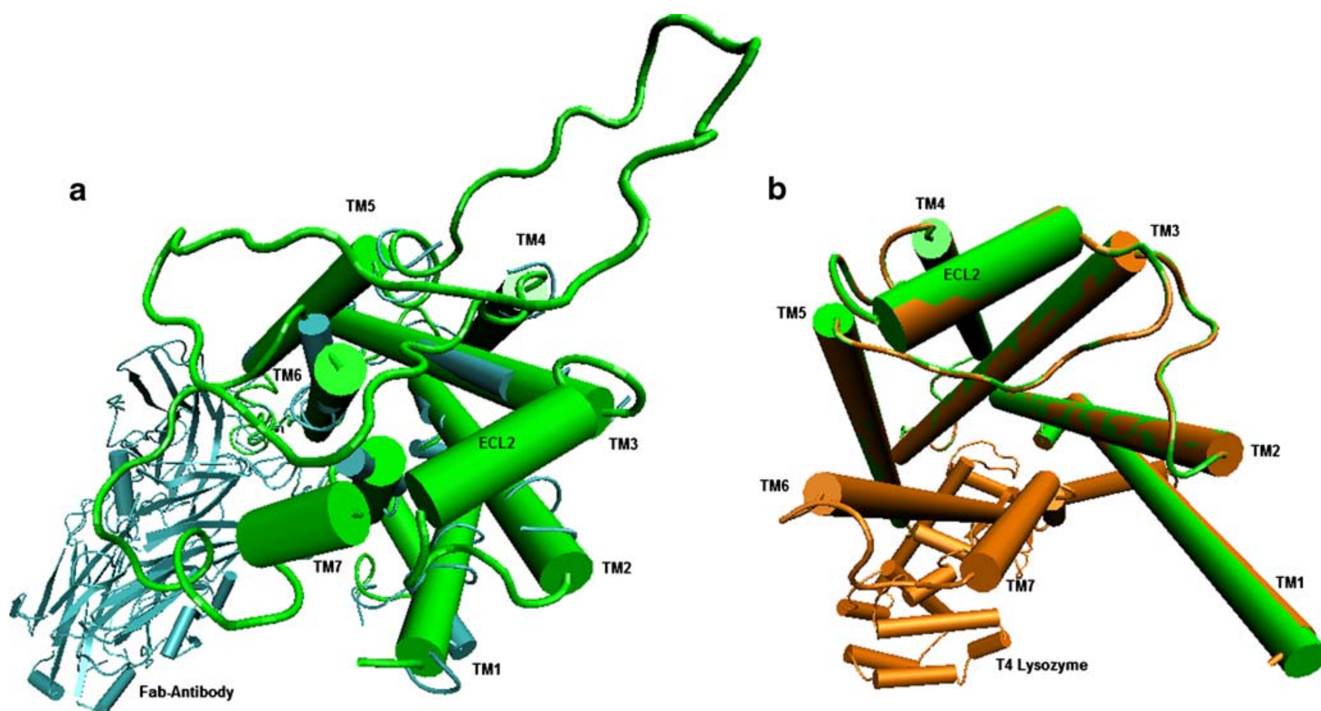


Fig. 1 Superimposition of the 3-D models obtained from Swiss-Model server (in green) compared with their template. **a.** Gβ_2AR 3-D model with seven transmembranal domains (7TM) obtained from model with PDB code: 2r4r (in cyan) as the template. **b.** Model with five transmembranal domains obtained using model with PDB code: 2rh1 (in orange) as the template

amino or carboxyl tails of the β_2AR [9] and they are neither included in experimental nor theoretical models.

Ligand binding on the Gβ_2AR

Docking simulations showed that the ligands were bonded at the Gβ_2AR binding site, which is similar to what was reported for the Hβ_2AR (Table 2) [10, 24–31]. The interactions with the residues take place at the transmembrane domains from 3 to 7 (Fig. 4). The theoretical pK_D values for the R-enantiomers of well-known agonists or antagonists tested showed a correlation with the in vitro reported values (Chart 2). The R^2 value calculated for ligands ordered with increasing pK_D was 0.869. This value is similar to the R^2 corresponding to ligands ordered by their affinity values (pD_2 or pA_2) reported in vitro (R^2=0.811).

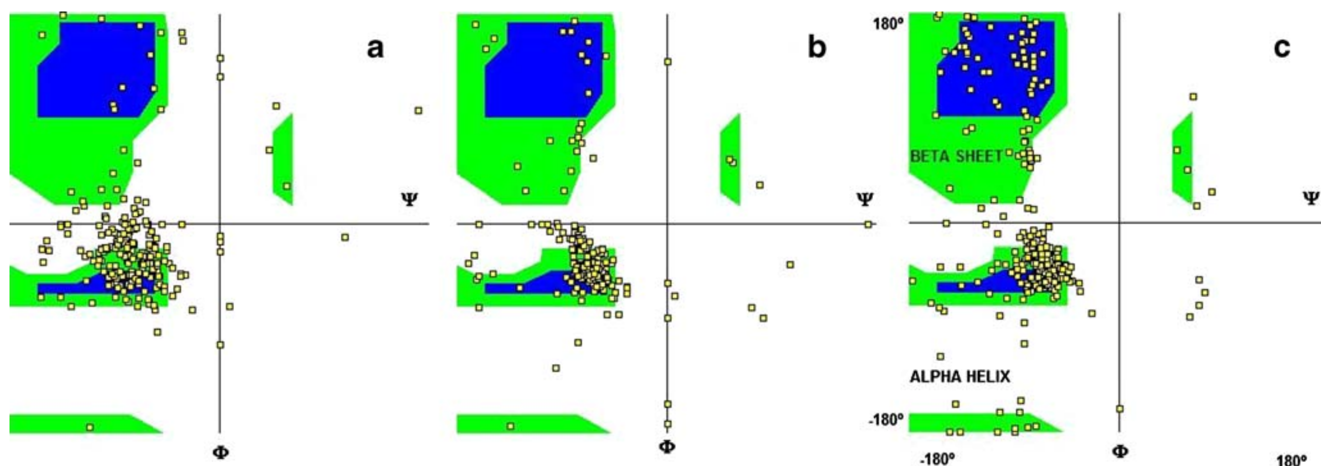


Fig. 2 Ramachandran plots generated by VMD 1.8.5 program, 3-D structure of the Hβ_2AR (PDB code: 2r4r) without structure minimization (**a**), Hβ_2AR after structure optimization with NAMD v.2.6 (**b**), Gβ_2AR after structure optimization with NAMD v.2.6 (**c**). White areas correspond to sterically disallowed regions, colored areas correspond to the generously allowed regions, blue areas correspond to the “core” regions representing the most favorable combinations of phi(Φ)-psi(Ψ) values

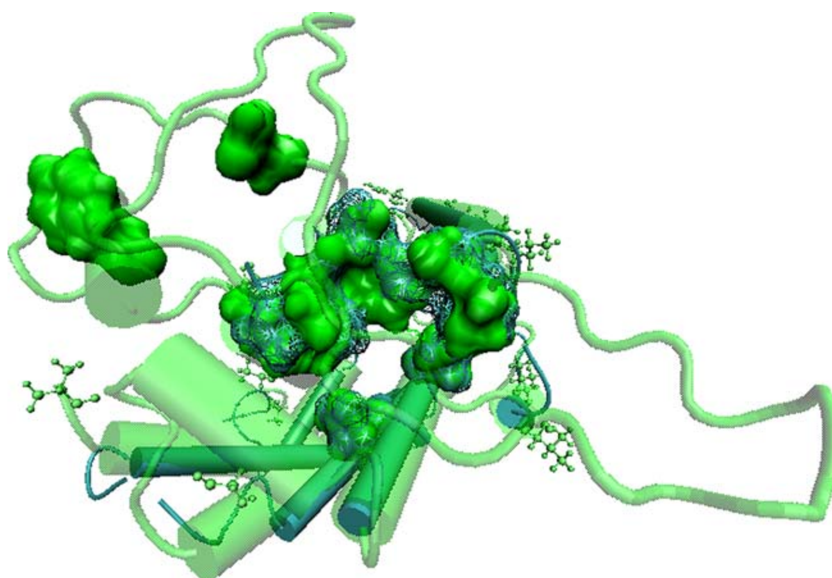


Fig. 3 Obtained $G\beta_2AR$ model superimposed on its template (PDB code: 2r4r), both optimized using NAMD v2.6 program. The residues reported as ligand binding site for $H\beta_2AR$ recognition and activation are represented as a green surface. Those for $G\beta_2AR$, cyan surface, wide for the $H\beta_2AR$ model. Additionally, the amino acids included in $G\beta_2AR$ that are different in $H\beta_2AR$ are in stick and ball representation

Therefore, the theoretical method reproduces experimental affinity values that are similar to the values determined by other well-established models [32]; whereas for the S-enantiomers there was no correlation. For 10 of 15 ligands tested, these S-enantiomers had a lower affinity for the $G\beta_2AR$ 3-D model compared with its corresponding R-enantiomer. This is a common phenomenon reported for in vitro assays [7, 20]. No predominant interactions with the specific amino acids involved in the enantioselectivity for the $H\beta_2AR$ (N293 or Y308) [33] have been described for the R or S ligand binding site on $G\beta_2AR$.

The pK_D values for R-Adrenaline, R-Noradrenaline and S-BR-AEA were most similar to the data obtained from in vitro assays [25, 27]. However, these values were treated as outliers in regression for correlation analysis, using methodology previous described [34]. All other ligands showed *in silico* affinity with the same trends that were reported in vitro, however, pK_D values were two orders of magnitude smaller than what was estimated by in vitro assays (Charts 1 and 2).

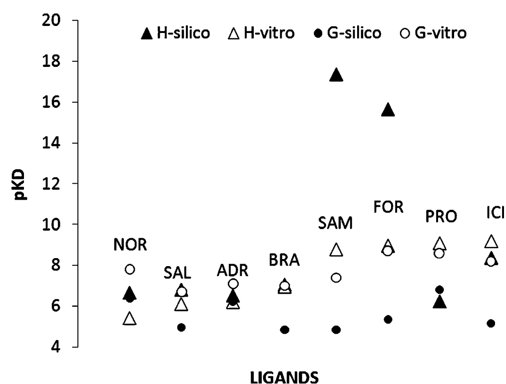


Chart 1 The ligand affinity values on the $G\beta_2AR$ under *in silico (black circles)* and in vitro experiments *(white circles)*, and the ligand affinity values on $H\beta_2AR$ under *in silico* (black triangles) and in vitro experiments *(white triangles)*. Note: Values obtained from *in silico* experiments are for the lowest free-energy complexes between R enantiomer ligands and β_2AR. See schemes for abbreviations

Table 2 Ligand affinities on the $G\beta_2AR$ calculated *in silico* and reported by experimental methods

Ligand	Calculated pK_D*		Reported pA_2 or pD_2 [Ref]
	R	S	
Atenolol	4.39	7.18	5.7 [24]
BR-AEA	4.84	7.0	7.0 [a] [23]
Salmeterol	***4.85***	4.79	7.4 [31]
Salbutamol	4.97	5.22	7.3 [a] [28]
ICI 118,551	5.15	5.27	8.2 [24]
Ritodrine	***5.25***	5.09	7.3 [27]
Formoterol	5.35	5.55	8.72 [28]
Carvedilol	***5.40***	5.12	8.71 [30]
Carazolol	***5.46***	5.36	8.9 [26]
TA2005	***5.70***	5.54	9.72 [28]
Adrenaline	***6.21***	6.0	7.11 [25]
Noradrenaline	***6.39***	6.32	7.8 [29]
Propranolol	***6.82***	6.68	8.6 [24]
Alprenolol	***6.89***	5.26	9.0 [26]
Pindolol	***7.09***	6.87	9.89 [27]
R^2	0.87[b]	0.5944[c]	0.81

* Mean of pK_D values for the five ligand-$G\beta_2AR$ complexes with lowest free energy. Values for R-enantiomers greater than is respective for S-enantiomers are in ***bold cursive***.

[a] In our in vitro studies BR-AEA showed greater affinity than salbutamol [23].

[b] The values for adrenaline and noradrenaline were considered outliner data.

[c] The values for atenolol and BR-AEA were considered outliner data.

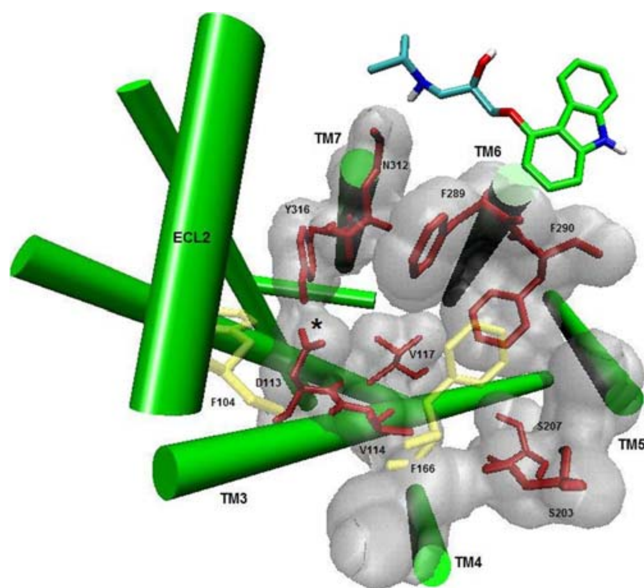


Fig. 4 The binding site for R-carazolol on the obtained Gβ$_2$AR 3-D. The residues interacting (with distances lower than 5 Å) are in bonds representation. Amino acids, colored in yellow, interact in this model but not in Hβ$_2$AR [9, 14]. Only alpha-helix residues are in cartoon representation. The asterisk shows the site for binding the amino group of all the ligands tested. The ligand was removed for clarity but its 3-D structure (optimized by Gaussian98 program at B3LYP 6–31G* level) is shown in the upper right corner. TM: transmembranal domain; ECL:extracellular loop

Ligand interactions on Gβ$_2$AR, observed by docking studies, were similar to what was described for Hβ$_2$AR. This confirms that the binding site is conserved, as was described by Kobilka, et al. [9, 14]. For a comparative example, R-Carazolol (on Gβ$_2$AR) interactions forms hydrophobic contacts with F104. The last amino acid interacts with the hydroxyl group by hydrogen bonding with D113, Y316 and N312 in its amine terminus. Other hydrophobic interactions were with the lateral chains of V114, V117, F166, F289 and F290. Lastly, a hydrogen bond formed between the lateral chain of S203 and the pendant amino in the pentaheterocyclic ring of carazolol (see Fig. 4).

Additionally, Kobilka, et al. described similar interactions for carazolol in crystallized Hβ$_2$AR [9, 14]. Two aromatic residues, F104 and F166, were important binding site components. Although, they do not have biological implications in in vitro studies, they might have an important role in the binding pocket formation. An example of this is the effect that W109 and F193 can have in the Hβ$_2$AR, which was first proposed by Rosenbaum et al. [14]. Both W109 and F193 are present in Gβ$_2$AR but were not included in the binding site for the ligands tested.

In Hβ$_2$AR, F193 contributes more buried surface area than any other residue to the interface between H Hβ$_2$AR and carazolol. Therefore, F193 is likely to contribute significantly to the energy of complex formation. The position of this residue on the extracellular side of the binding site may allow it to act as a gate. This would contribute to the unusually slow dissociation of the ligand [14]. Although F193 is outside of the binding site in Gβ$_2$AR, it can be substituted by F166 in the carazolol-Gβ$_2$AR complex (Fig. 4).

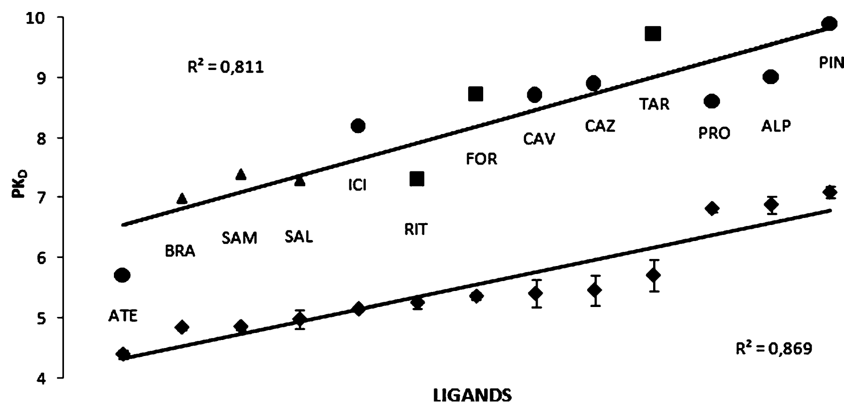
Another important amino acid of Hβ$_2$AR is W109, which shows hydrophobic interactions with the isopropyl moiety from the amino group of carazolol. These interactions are similar to those with F104 of Gβ$_2$AR.

Additionally, all ligands interacted with D113 and N312 of Gβ$_2$AR. The interaction was through hydrogen bonding with the amino group of the lowest conformational free-energy ligands. All other conformations docked in different sites. These sites are located on the lateral side of the receptor and are in contact with the bilayer lipidic membrane. Thus, these are not viable sites for interactions in a biologic system.

Conclusions

This work was aimed at determining a molecular model of the 3-D structure for Gβ$_2$AR. Theoretical affinity studies

Chart 2 pK$_D$ values calculated on the Gβ$_2$AR by *in silico* experiments and its correlation with in vitro reported data. Code: ♦ represents the mean of pK$_D$ values for the five complexes with lower free-energy. The bars are the standard error of mean in each case. ▲ = conventional agonist, ▪ = long action agonist and ● = inverse agonist or antagonist effect on the β$_2$AR. See schemes for abbreviations



with well-known ligands were used to compare the similarities and differences between this model and Hβ_2AR during the ligand recognition process.

We have obtained a Gβ_2AR 3-D model which showed it is capable for computational screening that can be used to carry out further computational screening as complementary tool for ligand behavior evaluation to in vitro tests in guinea pig models. In this 3-D model, the ligands (agonist or antagonists) interacted in similar form to one of the Hβ_2AR as have been identified experimentally. However, some differences were identified. Primarily, F104 and F166 are suggested to have a role in the binding pocket integration for the Gβ_2AR, which is similar to previously reported results with W109 and F193 in Hβ_2AR.

The docking simulations using R-enantiomers showed similar affinities than has been reported in vitro, and showed higher affinity than S-enantiomers in the majority of cases. However, no specific interactions with amino acids in the binding site were identified as contributing to this high affinity.

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