Letters in Applied NanoBioScience
Platinum Open Access Journal (ISSN: 2284-6808)

Research Article
Volume 10, Issue 1, 2021, 2063 - 2069
https://doi.org/10.33263/LIANBS101.20632069

An *In-Silico* Approach for Designing a Potential Antagonistic Molecule Targeting β_2-adrenoreceptor Having Therapeutic Significance

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Received: 17.09.2020; Revised: 14.10.2020; Accepted: 14.10.2020; Published: 17.10.2020

Abstract: One of the largest families of membrane proteins, the G protein-coupled receptors (GPCRs) has been a very important target of drug discovery as they are involved in having a regulatory role in a variety of signaling pathways at the cellular level in response to external stimuli. Modern in-silico and crystallographic approaches have further made it easier to peep into their structures. In this study, β2 adrenergic receptor (β2AR) has been targeted, and a new ligand molecule using the de-novo approach has been proposed. Using 1-Amino-3-(2,3-dihydro-1H-indol-4-yloxy)-propan-2-ol, the best fitting binding fragments were established with a significant dissociation constant value of 5-7 nanomolar. The flexibility of specific active sites was also investigated, and it was observed that residues 114 (V), 117 (V), 203 (S), 286 (W), and 289 (F) played a crucial role in accommodating ligand for the best binding. Upon examination of the bioavailability parameters, the ligand var9 exhibited significant inhibitory characteristics having lower toxicity values and high drug likeliness properties. Findings certainly hold significance in terms of targeting GPCRs in getting insight into structure-based drug designing and drug discovery.

Keywords: β_2-adrenoreceptor; G protein-coupled receptors; adrenoceptors; drug likeliness; toxicity; drug discovery.

1. Introduction

Adrenergic receptors or Adrenoceptors comprise of the largest family of cell surface receptors with about 700 genes in the genome, called G protein-coupled receptors (GPCRs), which are the potential targets of adrenaline and noradrenaline hormones formed in the body [1,2]. There are a number of cells that have adrenoceptors, which are known to stimulate the sympathetic nervous system (SNS). Adrenoceptors have also been shown to have an organ-specific response in humans for e.g., a rapid increase in the heart rate, energy metabolism, Diversion of the blood flow, etc. β-adrenergic receptors are of three subtypes, i.e., β1-ARs, β2-ARs, and β3-ARs, which facilitate extensive series of physiological reactions in our body [3-5]. β2 adrenoceptors are mainly associated with causing relaxation of smooth muscles in a variety of tissues. The β2 subtype has a high affinity for the endogenous agonist, adrenaline.

Synthetic β2 agonists include terbutaline, salbutamol, salmeterol, and zinterol, all of which have been proved therapeutically useful in the treatment of asthma and cancers as well

[6-9]. The varying levels of expression of $\beta_2$adrenoceptors have been reported in a number of species [10]. Novel drug molecules targeting GPCRs have been designed in the past, particularly the compounds such as β2 adrenergic receptors that respond to adrenaline and noradrenaline hormones. There are a variety of targets with reference to the currently available cardiac and asthma treatment drugs but are having a number of adverse side effects. Considering the above facts and with the rise in pollution levels and deteriorating environment, better quality drugs with improved drug likeliness properties are very much required. The exploration and retrieval of GPCR structures and simultaneously working upon them with modern drug designing approaches could certainly help in fetching improved drug compounds [11-12]. Therefore, considering the immense applications of such receptors in a broad range of disorders, in the present study β2 Adrenergic GPCR was chosen as our potential drug target [13].

2. Materials and Methods

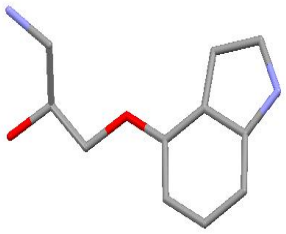
In-Silico ligand building has been performed using Ligbuilder [9] as we know that Ligbuilder is based upon a genetic algorithm that is known to construct a library of fragments on the basis of the target protein and its structural constraints. The coordinates of the seed molecule (in mol2 format) were used for the formation of a complete ligand simultaneously satisfying the active site or pocket of the receptor 2RH1. The seed molecule was grown into the pocket based on Lipinski's Rule of five [10].

Binding affinities of the populated ligands were estimated using the empirical scoring function. Using structural libraries, the chemical stability, feasibility, and toxicity of the molecules were considered. The binding energy of grown ligands was investigated using HEX[11]. The same program was used to perform the rigid docking and energy of each receptor-ligand complex and their interactions with Val114, Val117, Ser203, Phe290, Phe289, and Trp286. The selected binding pocket residues and the potent ligand cluster complex were visualized using RasMol [12]. The complexes with minimum energy were examined for their bioavailability, drug likeliness, and toxicity properties using Cheminformatics, Molinspiration [13] (drug-like properties, and bioactivity of the compounds), and Osiris Property Explorer (fitness of the ligand) [14].

3. Results and Discussion

In this study, the new molecule using the de-novo approach have been proposed targeting β2 adrenergic receptors (β2AR). With reference to its 3D structure obtained from PDB, the recruiting mechanism of amino acids at respective positions i.e., Va114, Va117, Ser203, Phe290, Trp286, and Phe289, were the prime step of the study. *In-Silico* ligand building was performed using Ligbuilder [15-16] in which more than 500,000 molecules were inhabited by consulting a library of organic fragments, for which we have used 1.2RH1.pdb (receptor) without heteroatoms and Ligand.mol2 (pre docked ligand, "Carazolol"), including hydrogen atoms as input file in processing the POCKET. We had an output file as: (1) 2RH1_grid.txt, (2) 2RH1_key_site.pdb, (3) 2RH1_pharmacophore.pdb, (4) 2RH1_pharmacophore.txt, and (5) 2RH1_pocket.txt. The seed molecule which we prepared was 1-Amino-3-(2,3-dihydro-1H-indol-4-yloxy)-propan-2-ol having the following properties: Molecular formula: $C_{11}H_{16}N_2O_2$, Molecular weight: 208.256 Da, logP:0.044 (Fig. 1). Using "1-Amino-3-(2,3-dihydro-1H-indol-4-yloxy)-propan-2-ol", the best fitting binding fragments

were established, with a significant dissociation constant value of 5-7 molars. The best molecule was obtained with docking energy values of 292.15 KJ/mol. The flexibility of specific active sites was also inspected, and it has been observed that residue 114 (V), 117 (V), 203 (S), 286 (W), and 289 (F) played a crucial role in accommodating ligand for best binding.

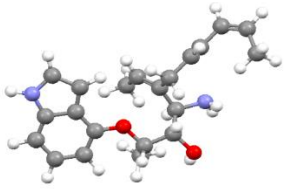
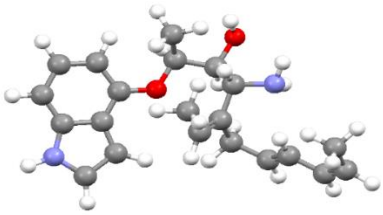
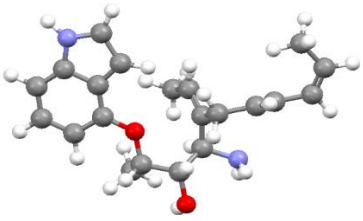
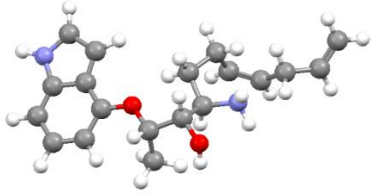
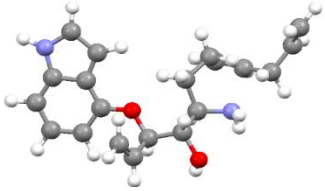


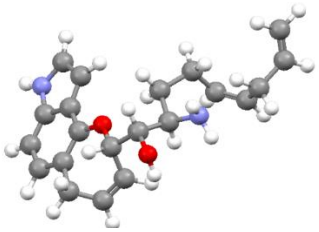
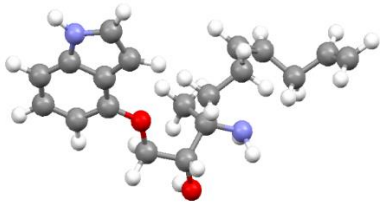
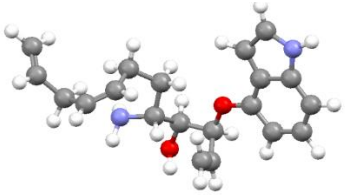
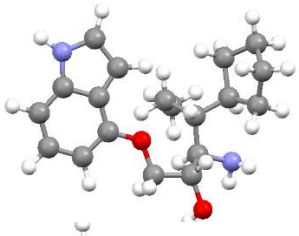
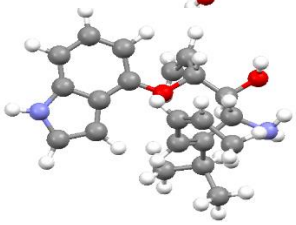
Stick view (without Hydrogen)

Figure 1. The seed molecule 1-Amino-3-(2,3-dihydro-1H-indol-4-yloxy)-propan-2-ol).

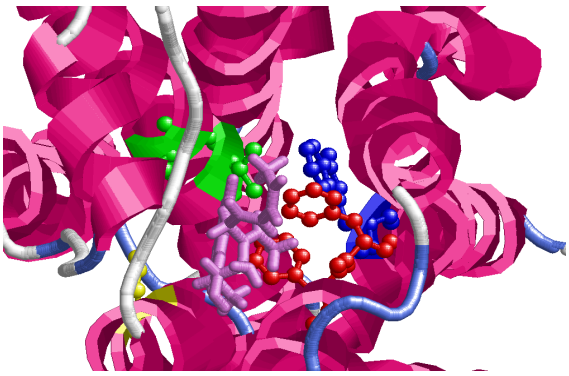
The coordinates of the seed molecule (in mol2 format) were used further for the formation of a complete ligand simultaneously satisfying the active site or pocket of the receptor 2RH1. Using the growing strategy (input used is: Seed.mol2 while the output as (1) Ligands_2RH1.lig and (2) Population_2RH1.lig), the seed molecules obtained were processed, generating 10 resultant molecules out of thousands of proposed ligands as shown in Table 1.

Table 1. The resultant ligands were obtained after satisfying the active site or pocket of the receptor 2RH1.

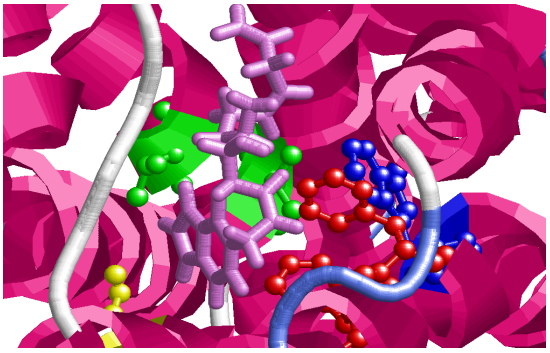
Result No.	Structure (Ball & Stick view)	Formula Weight
1.		342 Da
2.		341 Da
3.		340 Da
4.		315 Da
5.		328 Da

Result No.	Structure (Ball & Stick view)	Formula Weight
6.		340 Da
7.		322 Da
8.		328 Da
9.		318 Da
10.		348 Da

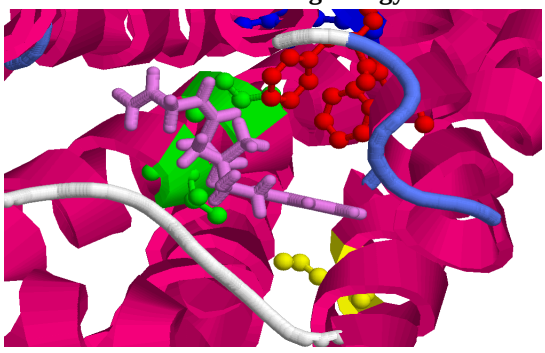
The empirical scoring function was used to estimate the binding affinities of the populated ligands. Other properties like chemical stability, feasibility, and toxicity were also considered using structural libraries. After screening, a total of 10 molecules were selected for further study. The binding energy of grown ligands was investigated using HEX[17]. The same program performed the rigid docking and energy of each receptor-ligand complex, and their interactions with Val114, Val117, Ser203, Phe290, Phe289, and Trp286 were further evaluated with the number of clusters with different energies as an output. Minimum energy clusters were chosen out of each docked complex. The binding pocket residues and the potent ligand cluster complex selected were further visualized using RasMol [18]. The ligands showing maximum interactions and minimal energy were chosen as the potential ligands against β_2AR. The best-docked cluster complexes have been shown in figure 2 where the ligand is shown in violet while the residues Phe, Trp, Ser, and Val. identified on β2GPCR l, have been shown in red, blue, yellow, and green colors, respectively. Our ligand binding site has been shown in which the groove is formed by the seven helices of the β2AR structure**.**



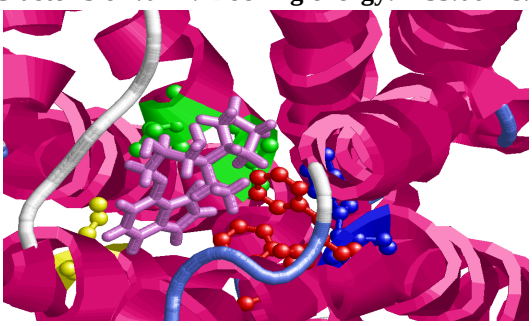
(a) Cluster 1 of var 3:- Docking energy: -285.7 KJ/mol



(b) Cluster 14 of var 6:- Docking energy: -224.09 KJ/mol



(c) Cluster 3 of var 7:-Docking energy: -233.06 KJ/mol



(d) Cluster 1 of var 9:- Docking energy: -292.15 KJ/mol

Figure 2. Four of the best-docked cluster complexes. Ligand is shown in violet while the residues Phe, Trp, Ser, and Val. identified on β_2GPCR l are shown in red, blue, yellow, and green, respectively. The ligand-binding site is formed by the seven helices of the β_2AR structure.

The complexes resulted in minimum energy were further examined for their bioavailability, drug likeliness, and toxicity properties using Cheminformatics, Molinspiration [19], and Osiris Property Explorer [20]. We observed through this analysis that 1st cluster of result 9 obtained from Hex was having high drug likeliness, higher drug score, and low toxicity while other parameters were the logP value of this compound as 2.32 with a molecular weight of 316.45 Da. (Fig. 3). Therefore the molecule appears to be an abiding molecule since it fulfills

all the conditions which a ligand must in order to qualify for being used as a potent drug molecule.

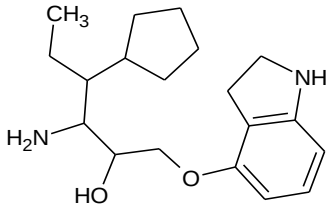


Figure 3. The structure of the potent drug molecule ($C_{19}H_{30}N_2O_2$).

After assessing the bioavailability parameters, the var9 (ligand) exhibited significant β_2AR inhibitory characteristics with lower toxicity levels but having high drug likeliness properties. Var9 (ligand) exhibited the enzyme inhibition values of 0.30, making Var9 (ligand) to be the best potent antagonistic β_2AR ligand molecule.

4. Conclusions

G protein-coupled receptors (GPCRs), one of the largest family of membrane proteins, have been extremely important targets from the drug discovery perspective. These proteins have a variety of regulatory roles in cellular signaling pathways. The in-silico modern approaches and crystallographic analysis have been instrumental in determining their structures as well. β2 adrenergic receptor (β2AR) was targeted in the present study leading to the designing of an antagonistic ligand molecule using the de-novo approach. The best-fitting binding fragments were further established with a significant dissociation constant value of 5-7 nanomolar. The flexibility of specific active sites was further investigated with the crucial role played by 114 (V), 117 (V), 203 (S), 286 (W), and 289 (F) residues in accommodating the ligand for the best binding. The ligand var9 exhibited significant inhibitory characteristics having lower toxicity values and high drug likeliness properties, making this as a biocompatible molecule. Further studies are warranted to study this molecule in vitro and in-vivo so as to establish the therapeutic significance of the molecule. The study certainly holds significance in targeting GPCRs so as to get mechanistic insight into novel drug discovery and structure-based drug designing.

Funding

This research received no external funding.

Acknowledgments

We greatly acknowledge Maharishi Markandeshwar (Deemed to be University) Mullana (Ambala) Haryana, India, for providing the requisite facilities to carry out the said work.

Conflicts of Interest

The authors declare no conflict of interest.

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