Revolutionizing Asthma Treatment: A Breakthrough in LABA Design Enhances Both Selectivity and Efficacy

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Abstract

Asthma, which affects 262 million patients globally, presents a significant health concern with substantial economic ramifications. Despite serving as the frontline treatment, current long-acting β2-agonists (LABAs) often sacrifice efficacy or selectivity for the other due to the decades-long challenge posed by the highly conserved ligand-binding sites shared between the target receptor and the off-target receptor, namely β2-adrenergic receptor (β2AR) and β1-adrenergic receptor (β1AR). Observing that conventional virtual screening approaches are ineffective, this computational study proposes a new method and addresses the challenge. By combining structural groups with consideration of known connections between LABAs' structural groups, receptor interactions, and physiological effects, this study created a novel analog of LABA that is not only the first to be selective and fully efficacious but also the first to perform existing LABAs in both selectivity and efficacy *in silico*.

1. Introduction

Affecting 262 million patients and leading to 450,000 deaths in 2019, asthma is the most common noncommunicable disease worldwide and is economically burdensome, incurring a cost of $81.9 billion in the United States alone annually (Global Initiative for Asthma, 2022; Nurmagambetov et al., 2018).

1.1 Mechanism of Action of β2-agonists

β2-agonists are a class of drugs that constitute the frontline treatment for asthma. When asthma patients are exposed to allergens or other stimuli, airway epithelial cells release inflammatory molecules that trigger airway smooth muscle (ASM) contraction and lead to airway constriction (Tanabe et al., 2007; Lambrecht et al., 2019; Yamauchi & Ogasawara, 2019). β2-agonists target β2 adrenoceptors (β2ARs) of the GPCR family on ASM cells and, when bound, trigger the α subunit of the G-protein (Gαs) to detach, activating adenyl cyclase and protein kinase A (PKA) (Fig. 1) (Tiwari & Gupta, 2021; Billington et al., 2016). Through regulating intracellular calcium levels, PKA leads to ASM relaxation and bronchodilation.

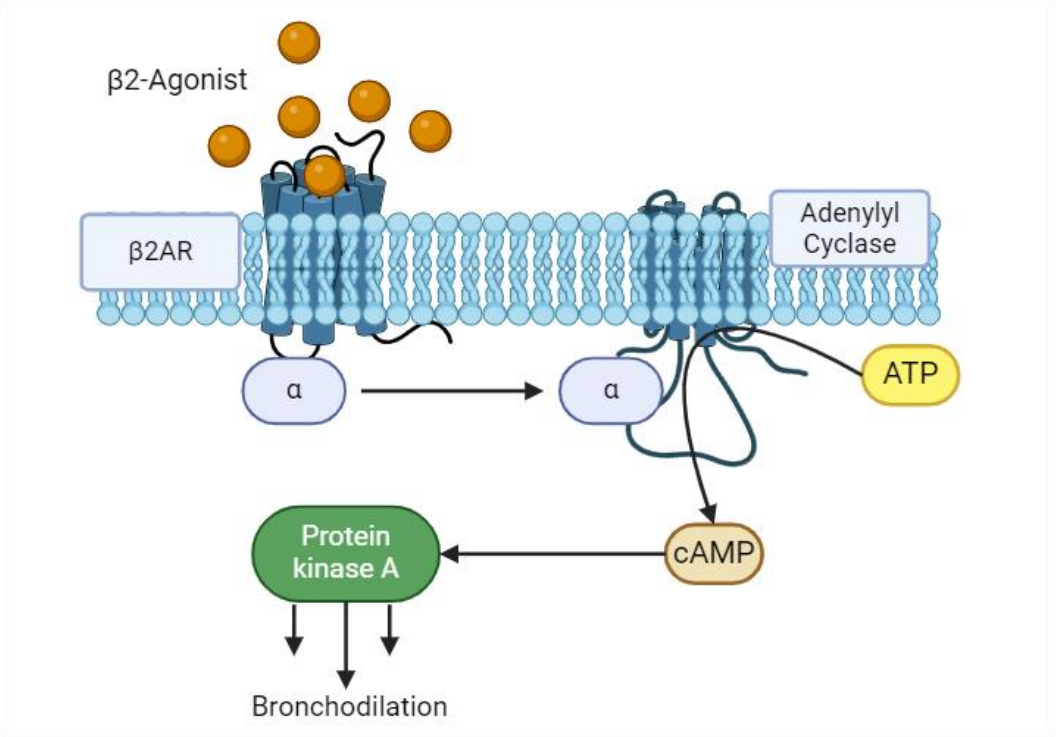
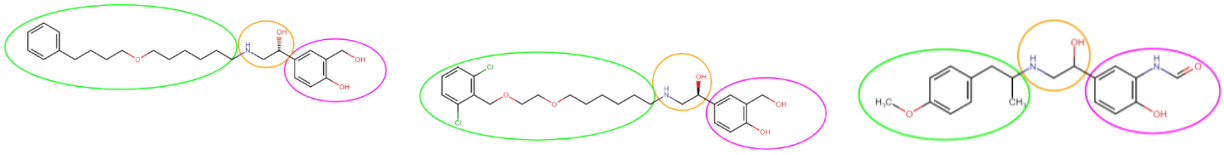


Figure 1. *Mechanism of β2-agonists (Billington et al., 2016).*

1.2 β2-agonists Classifications: LABAs

β2-agonists are categorized by their duration of action. The relevant one to the study is long-acting β2-agonists (LABAs), which are long-term control medications that relieve symptoms for up to 12 hours. LABAs approved for asthma treatment are salmeterol, formoterol, and vilanterol (Fig. 2).



Salmeterol **Vilanterol** **Formoterol**

Figure 2. *Chemical structure of salmeterol, vilanterol and formoterol. Magenta represents head group, orange represents ethanolamine group and green represents tail group.*

1.3 The Need for a New Analog

While widely used, LABAs do not exhibit high receptor selectivity and, at times, lead to "off-target" cardiovascular effects by unintentionally stimulating β1AR, doubling users' risk of myocardial infarction, cardiac arrest, and sudden cardiac death, making LABAs unsuitable for patients with cardiovascular diseases (Billington et al., 2016; Cazzola et al., 2005).

The highly conserved ligand-binding site shared between β1AR and β2AR impedes the development of better LABAs. The structures of a turkey β1AR and a human β2AR deviate by merely 0.58 Å, and the gene sequences of β1AR and β2AR are 92% similar (Masureel et al., 2018). Moreover, the orthosteric ligand-binding pockets of the receptors are identical except for Tyr308 (Woo et al., 2014).

Among LABAs, salmeterol and vilanterol have the highest selectivity for β2AR over β1AR and pose the least cardiovascular risks (1000-fold for β2AR over β1AR) (Baker et al., 2014; Cazzola et al., 1998). However, they also have weaker binding affinity to the β2AR and fewer hydrogen bonds to activate β2AR, making them partial agonists with lower intrinsic efficacy and weaker bronchodilation. In contrast, the β2-agonist with the highest intrinsic efficacy is formoterol, a full agonist that binds strongly to β2AR with strong, abundant hydrogen bonds to activate β2AR. Nonetheless, it has low selectivity (200-fold for β2AR over β1AR) (Naline et al., 1994; Cazzola et al., 1998; The Ritedose Corporation, 2019). As current LABAs sacrifice either high efficacy or selectivity for the other, a new analog of LABA providing both qualities is needed to increase patient safety, applicability, and drug adherence.

1.4 Overcoming the Challenges of Selectivity and Efficacy

A list of patterns that will enhance drug design has been compiled based on past research that studied the connection between LABAs' structural groups, receptor interactions, and physiological effects.

1.4.1 Basic Scaffold of LABAs Determines Activation and Duration of Action

All LABAs approved for asthma treatment consist of a head group (an aromatic ring with hydrogen bond donors and acceptors), an ethanolamine group, and a tail group (a carbon chain linked to an aromatic ring) (Fig. 2). This is because all LABAs occupy the same orthosteric ligand-binding pocket of β2AR located within β2AR's seven-transmembrane helices (7TMs). The common scaffold also prolongs the duration of action of the LABAs. Their lack of a catechol head group prevents them from being quickly metabolized by catechol-*O*-methyltransferase. At the same time, their adequately hydrophobic tails allow them to be incorporated into the lipid bilayer near the receptor to maintain a high concentration of the drug on the cell membrane (Waldeck, 2002; Anderson et al., 1994). Regarding salmeterol and vilanterol, hydrophobic interactions between the exosite and the tail group also help anchor the LABAs in the vicinity of β2AR after dissociation and help make them available for rebinding (Coleman et al., 1996; Szlenk et al., 2021).

1.4.2 Head Group Interactions Determine Efficacy

A LABA's head group must have strong hydrogen bond interactions with Ser203 and Ser207 to ensure full agonism. When full agonist epinephrine (Fig. 3a), which is the endogenous ligand of β2AR, or full agonist formoterol (Fig. 3c, 3d) binds to the orthosteric ligand-binding pocket, their head groups form stabilizing hydrogen bonds with the two serine residues, which causes TM5 to move inward and the cytoplasmic end of TM6 to move outward, thus activating β2AR (Masureel et al., 2018; Szlenk et al., 2021). In contrast, the head group of partial agonist salmeterol forms hydrogen bonds that are weak at stabilizing the inward movement of TM5, as evidenced in the reduced number of residues on TM5 within 4Å of salmeterol during a past study of molecular dynamics simulation (Fig. 3b and 3e) (Masureel et al., 2018; Szlenk et al., 2021). Thus, salmeterol can only partially activate β2AR.

The structure of vilanterol-bound β2AR has not been found. However, the shared head group of vilanterol and salmeterol suggests that the above explanation most likely applies to vilanterol's partial agonism.

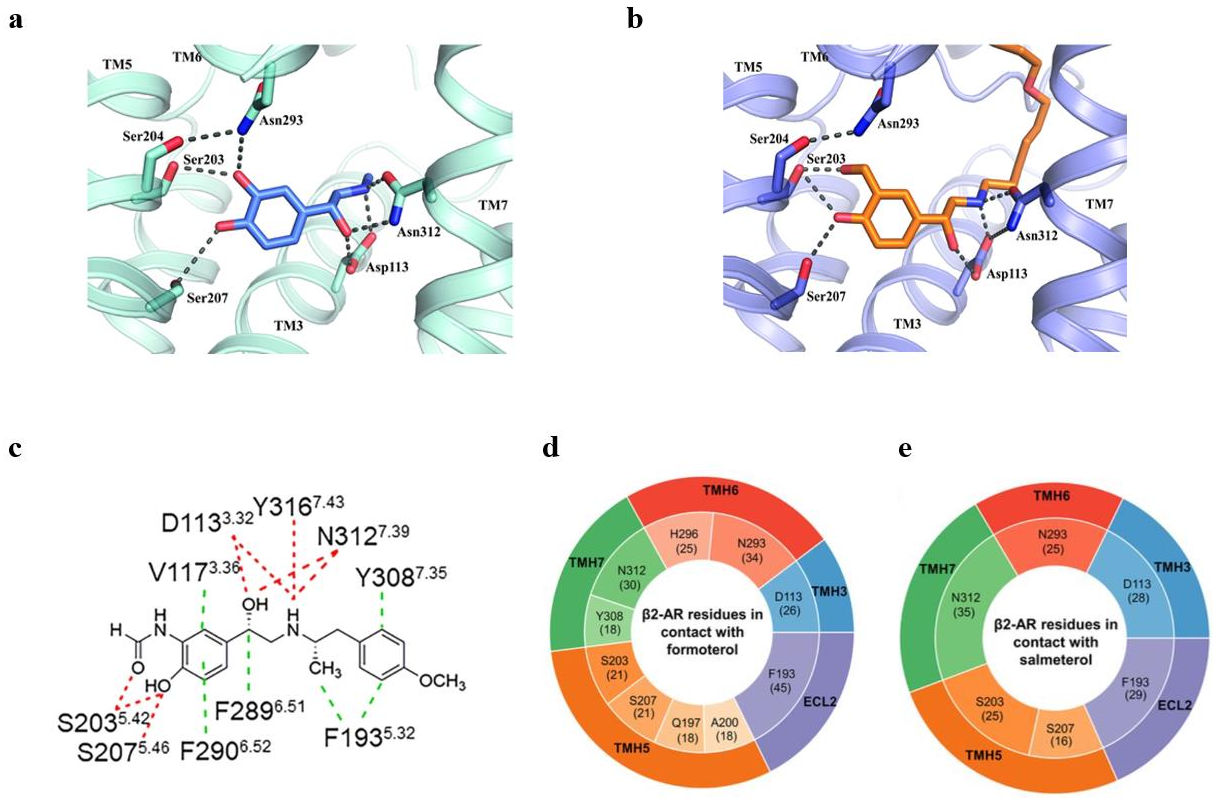


Figure 3. *(a-c) Binding map of epinephrine, salmeterol, and formoterol (Masureel et al., 2018; Zhang et al., 2020). Red represents hydrogen bonding and polar interaction, while green represents hydrophobic interaction. (d-e) Pie charts representing residues from various transmembrane helices (TMHs) and extracellular loop 2 (ECL2) and percentage of occupancy within 4Å of formoterol and salmeterol (Szlenk et al., 2021).*

1.4.3 Head Group Interactions Determine Subtype Selectivity

β2AR selectivity is achieved through binding to the exosites of β2AR via π interactions. In the case of salmeterol and vilanterol, hydrogen bonding of the oxygen atom in the tail group with the Phe193 allows the tail to bend, fit in the exosite, and engage in π interactions with the nearby residues Phe194, Tyr308, and His296 (Fig. 4) (Masureel et al., 2018). In the exosite of β1AR, the two aromatic residues Phe194 and His296 are replaced by nonaromatic residues Asp313 and Val202, which weakens salmeterol's and vilanterol's binding affinity for β1AR, allowing them to be highly selective for β2AR (Masureel et al., 2018; Wendell et al., 2019).

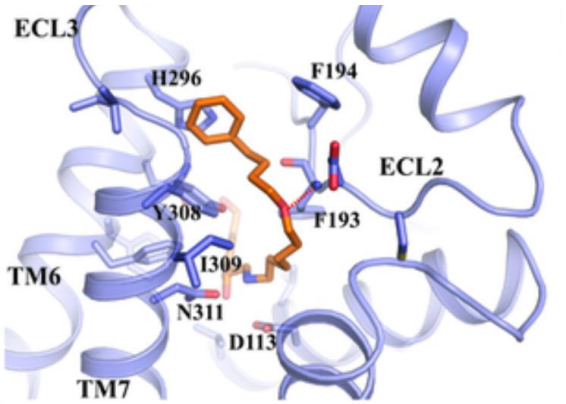


Figure 4. *Binding map of salmeterol in β2AR's exosite (Masureel et al., 2018).*

1.4 Statement of Purpose

This study hopes to design the first selective and fully efficacious analog of LABA by combining structural groups of different approved LABAs based on observations above.

2. Materials and Methods

2.1 General Materials

This study used Avogadro (version 1.2) (Hanwell et al., 2012), UCSF Chimera (version 1.17.3) (Petersen et al., 2004), AutoDock Vina (version 2) (Goodsell, 1990), Chemaxon MarvinSketch (version 19) (Chemaxon, 2020), Discovery Studio Visualizer (BIOVIA, 2024), RCSB PDB, and SwissADME (Daina et al., 2017).

2.2 Protein Preparation

The crystal structure of human β2AR bound to salmeterol and Nb71 (PDB ID: 6MXT) and the crystal structure of activated turkey β1AR with bound agonist formoterol and nanobody Nb80 (PDB ID: 6IBL) were imported to the UCSF Chimera from the Protein Data Bank as a *pdb. file. Hydrogen atoms and charges were added to the protein to investigate intermolecular forces, and the solvent was removed to reduce computational time. For β2AR, residues (H2O, T2O, P33, OLA, and OLC) and chain N were removed to increase computational efficiency. For β1AR, residues 2CV, H2O, chains B, C, and D were removed. The dock-prepped proteins are saved as *mol. and the sessions as *py. files.

2.3 Molecular Docking Experiment of Control Groups

Based on results from x-ray diffraction of human β2AR bound to salmeterol and Nb71, the binding site selected for docking for human β2AR was confined to a box with center at -8.9, -2.48715, 39.0456 and size of 16.4195, 14.641, 19.9925 (Masureel et al., 2018). Based on results from x-ray diffraction of activated turkey β1AR with bound agonist formoterol and nanobody Nb80, the binding site selected for docking for β1AR was confined to a box with center -

42.7856, 28.2359, -13.9716 and size of 18.8534, 13.031, 21.5095 (Lee et al., 2020). For each control group (salmeterol, formoterol and vilanterol) the *pdb. file of the LABAs was imported to UCSF Chimera and AutoDock Vina. Each control group was docked to each protein, β2AR and β1AR, for five trials. The conformation with the most negative ΔG of each trial was selected for further analysis.

2.4 Analog Design and Molecular Docking Experiment of Analogs

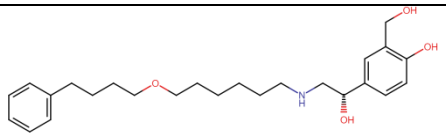
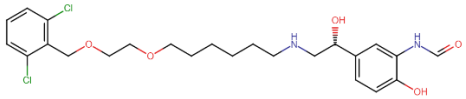
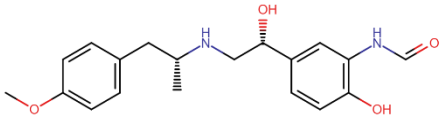
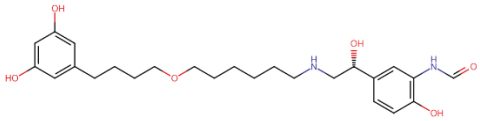
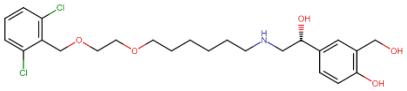
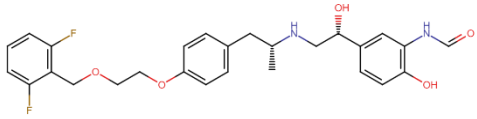
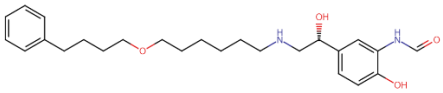
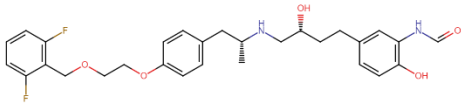
Two-dimensional structures of novel analogs of LABA were designed with Chemaxon MarvinSketch and saved as *mol. files. The files were then imported to Avogadro and converted into three-dimensional structures. Molecular geometries were optimized based on the universal force field (UFF). The analogs were exported as *pdb. files and SMILES files. The analogs' SMILES files were imported into SwissADME to analyze the analogs' drug-likeness using Lipinski's rule of five: molecular weight, logP, number of rotatable bonds, number of hydrogen bond acceptors and donors, and surface area. Analogs that did not fulfill the rule were discontinued from the procedure. The *pdb. file of each analog was imported to UCSF Chimera and AutoDock Vina. Each control group was docked to each protein, β2AR and β1AR, for five trials. The conformation with the most negative ΔG of each trial was selected for further analysis.

2.5 Data Analysis

Seven t-tests were performed to identify any statistically significant differences (1) between individual ligand's ΔG for β2AR and β1AR, (2-4) between analog and control groups' ΔG for β2AR, and (5-7) between analog and control groups' ΔG for β1AR. The binding position with the most negative ΔG among five trials, which is most energetically favorable and thus most likely to happen, was analyzed. Chimera was used to identify hydrogen bonds, clashes, and contacts. Discovery Studio Visualizer was to find other types of intermolecular forces and to generate 2d interaction maps. These, combined with the ΔG, are recorded and analyzed in the context of past literature (Masureel et al., 2018). Steps mentioned in 2.4 and 2.5 are repeated until the analog is shown to be fully efficacious and selective.

3. Results and Discussion

Table 1: Chemical structures of salmeterol, formoterol, vilanterol and some of the analogs

Salmeterol		Analog 20	
Formoterol		Analog 67	
Vilanterol		Analog 71	
Analog 2		Analog 73	

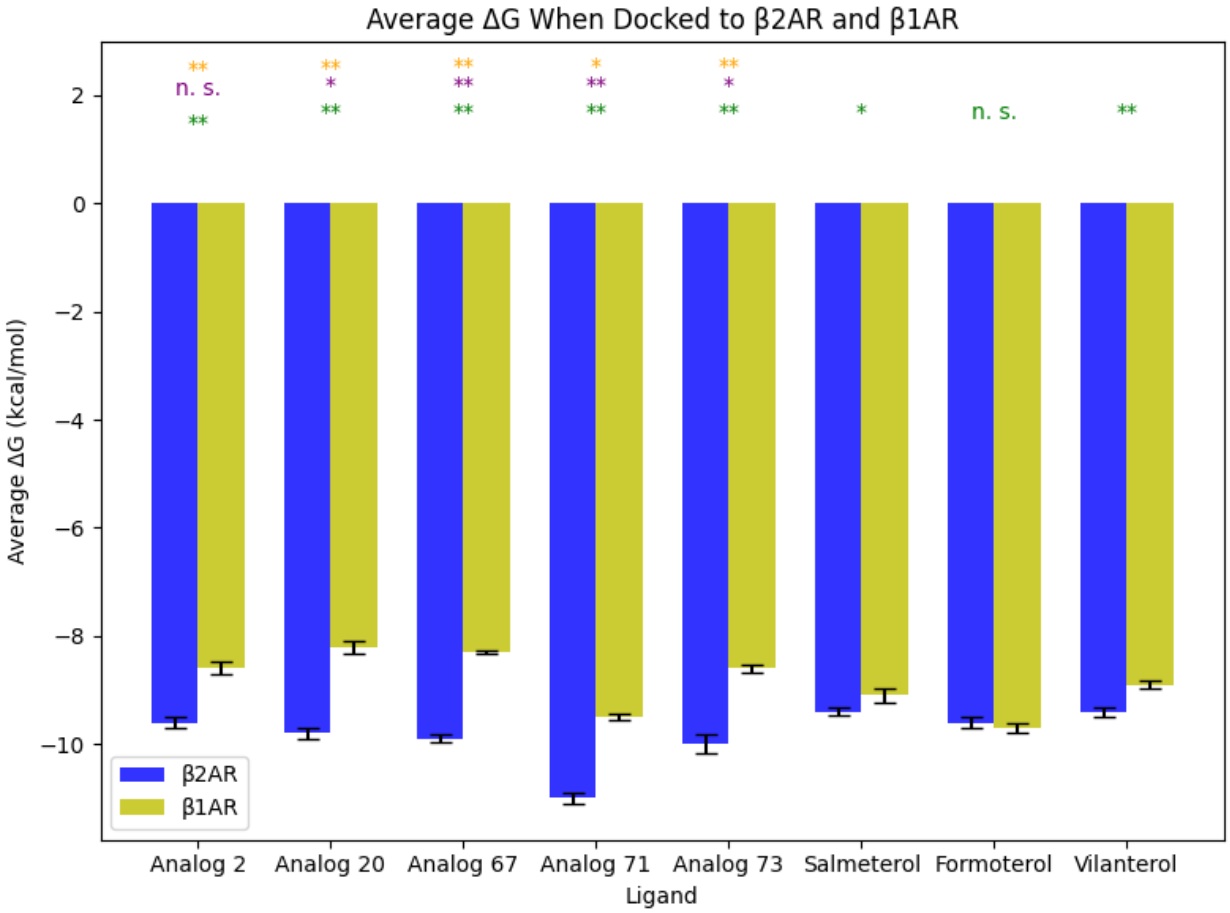


Figure 5. *A graph showing the average ΔG when each ligand is docked to β2AR and β1AR. Green represents t-test comparing each ligand's ΔG for β2AR and β1AR. Purple represents t-test comparing each ligand's ΔG to the control groups for β2AR. Yellow represents t-test comparing each ligand's ΔG to the control groups for β1AR.*

Table 2: Quantitative Analysis of Control and Analogs[1]

Ligand	ΔG in β2AR (kcal/mol)	ΔG in β1AR (kcal/mol)
(R,R)-Formoterol	-9.7	-9.8
(R)-Salmeterol	-9.5	-9.3
(R)-Vilanterol	-9.5	-9.0
Analog 2	-9.8	-8.8
Analog 20	-9.9	**-8.3**
Analog 67	-10.0	**-8.3**
Analog 71	**-11.2**	-9.6
Analog 73	-10.2	-8.7

Table 3: Qualitative Analysis of Control and Analogs in β2AR

Ligand	Hydrogen Bond AAs	Clashes AAs with Overlap Angle (Å)	# of Contacts
(R)-Salmeterol	Phe193, Ser203, Asn312	Asn312 (0.966), Asn312 (0.65)	146
(R,R)-Formoterol	Ser203(x2), Ser207, Asn312(x3), Asn293	Asn312 (0.624)	108
(R)-Vilanterol	Asp113(x2), Ser203, Ala200, Phe193	None	144
Analog 2	Asp113, Ser203, Ans293, Asn312	None	146
Analog 20	Ser203, Ser207, Asn293, Asn312, Asp113, Phe193	None	155
Analog 67	Asn293, Asn312, Ser203, Arg304, His296	None	167
Analog 71	Asn293, Ser203(x2), Asp113(x2), Asn312, Phe193	None	162
Analog 73	Ser203, Asp113(x2)	None	137

[1] The binding position with the minimum ΔG is used for analysis because it is the most energetically favorable and thus it is most probable binding position.

Table 4: Qualitative Analysis of Control and Analogs in β1AR

Ligand	Polar-Interactions AAs	Clashes AAs with Overlap Angle (Å)	Contacts AAs
(R)-Salmeterol	Ser211, Ser215, Asn329	Val326 (0.727), Asp121 (0.647)	176
(R,R)-Formoterol	Ser211 (x2), Asn310, Asn329	Asn329 (0.639)	103
(R)-Vilanterol	Ser211, Trp330	Phe325 (0.636)	144
Analog 2	Asp121, Ser211, Ser215, Asn329	None	129
Analog 20	Ser211(x2), Asn310, Asp121	Phe325 (0.683), Val122 (0.682), Asn329 (0.627)	158
Analog 67	Ser215(x2), Thr126, Asn329, Asp121	None	148
Analog 71	Asp121, Ser211	Val122 (0.799)	180
Analog 73	Asn310, Asp121	None	168

3.1 Molecular Docking Analysis of Control Groups

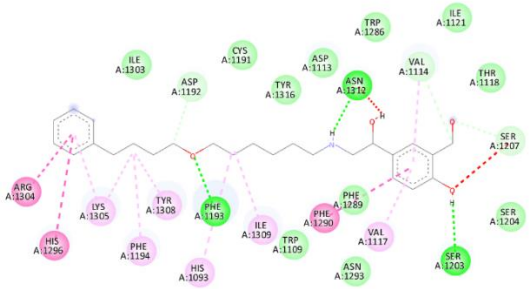
Molecular dockings of the control (r)-salmeterol, (r,r)-formoterol, and (r)-vilanterol showed that most of the critical bonds in the literature were maintained. When docked to β2AR, salmeterol formed hydrogen bonds with Ser203, Phe193, and Asn312 as in past studies (Fig. 6a). The absence of hydrogen bonds with Ser207 and Asn293 aligns with past findings that these interactions are unstable and account for salmeterol's partial agonism. Vilanterol exhibited almost the same interactions except its head group hydrogen bonded with Ala200 and its ethanolamine group with Asp113 (Fig. 6b). For formoterol, hydrogen bonding with Ser207, Asn293, Asn312 and Asp113 appeared as in previous studies (Fig. 6c). Formoterol has a more negative ΔG (-9.7 kcal/mol) and higher number of hydrogen bonds than salmeterol and vilanterol (-9.5 kcal/mol), which aligns with its being a full agonist. It is important to note that a difference of -0.2 kcal/mol is huge because it represents -200 cal/mol and translates to formoterol having 1.8-fold higher binding affinity for β2AR than salmeterol and vilanterol.[2]

When docked to β1AR, salmeterol hydrogen bonds with Ser211, Asp121 and Phe201 with two clashes: Asp121 with 0.647Å overlap and Asp121 with 0.647Å overlap (Fig. 6d). For vilanterol, hydrogen bonds with Ser211 and Trp330 were observed, so was a clash with Phe325 with 0.636Å overlap (Fig. 6e). For formoterol, there were hydrogen bonds with Ser211, Asn329 and Asn310 as well as a clash with Asn329 with 0.639Å overlap (Fig. 6f). Unlike the other two LABAs, there was no statistically significant difference between formoterol's ΔG for β2AR and β1AR. As more hydrogen bonds and fewer clashes were observed in formoterol when docked to

[2] $\Delta G = -RTlnK_{eq}$ where G is the Gibbs free energy or ΔG, RT is fixed at .5 kcal/mol in AutoDock Vina, and K_{eq} represents $[product]/[reactant]$ at equilibrium, which is the measure of binding affinity in folds.

β1AR, formoterol has a more negative ΔG (-9.7 kcal/mol) than salmeterol and vilanterol (-9.3 kcal/mol, -9.0 kcal/mol).[3] With strong conformation to previous *in vitro* and *in vivo* studies, the docking results of the controls lay a solid basis for testing analogs' efficacy and selectivity.

a[4]



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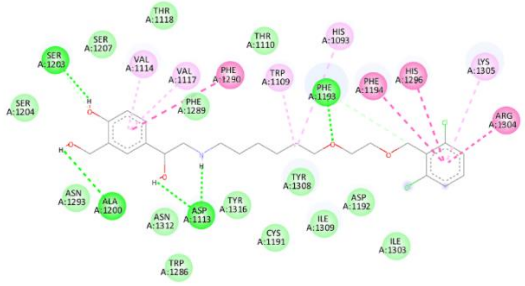

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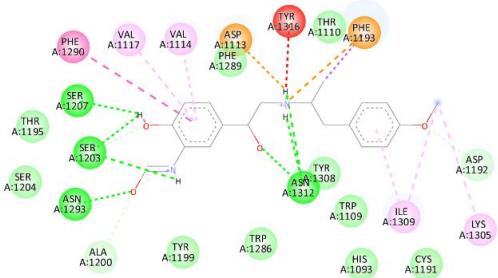

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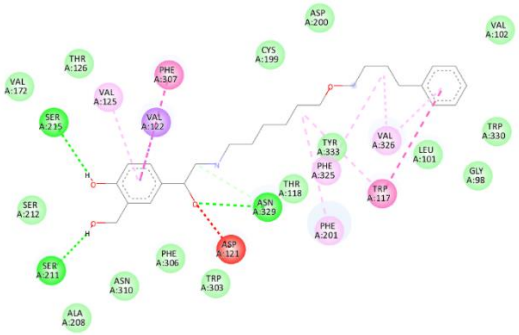

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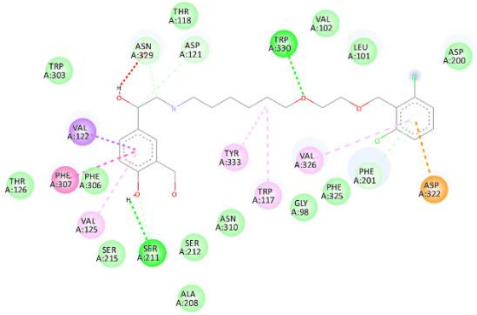

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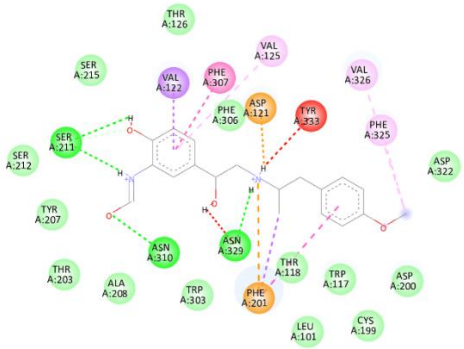

g

[3] There is no statistically significant difference between ΔG of salmeterol and vilanterol when docked to β1AR.
[4] Due to the protein being sequenced from biochemical assays, the numbering of residues in β2AR is 1000 more than the real values. Numbering in β1AR was adjusted and thus remains accurate.

Figure 6. *(a-c) The binding map of salmeterol, vilanterol and formoterol to β2AR, (d-f) and β1AR. (g) Legend.*

3.2 Molecular Docking Analysis of Analogs

Based on the molecular docking results and previous research results, formoterol's head group is essential in being a full agonist. At the same time, salmeterol's and vilanterol's tails are crucial to its high subtype selectivity. To create an analog that is selective and fully efficacious, a total of 76 analogs have been made by combining formoterol's, salmeterol's, and vilanterol's structural groups to various extents and making modifications to enhance binding. Five selective analogs, exhibiting more negative ΔG when bound to β2AR and less negative ΔG when bound to β1AR, are chosen to be elaborated below.

3.2.1 Analog 2

Analog 2 comprises formoterol's head group, an ethanolamine group, and salmeterol's tail group (Table 1). When docked to β2AR, hydrogen bonds with Ser203, Asn293, Asp113, and Asn312 were observed, but hydrogen bond with Ser207, which is critical to full β2AR activation, was missing (Fig. 7a). Hydrogen bond with Phe193 was absent. However, the phenyl tail still engaged in π interactions with aromatic residues at the exosite. With no clashes and an abundance of contacts (Table 3), analog 2 has a statistically significantly more negative ΔG (-9.8 kcal/mol) than salmeterol and vilanterol (-9.5 kcal/mol, p-value<0.05). However, due to the incomplete head group interactions, it did not have a statistically significant difference in ΔG than formoterol. When docked to β1AR, hydrogen bonds with Ser211, Ser215, Asp121, and Asn329 were observed (Fig. 7b). Reduced number of contacts (Table 4) and interactions within 4Å (Fig. 7b) likely led the ΔG of analog 2 to be less negative than that of the control groups (-8.8 kcal/mol, p-value < 0.01).

a **b**

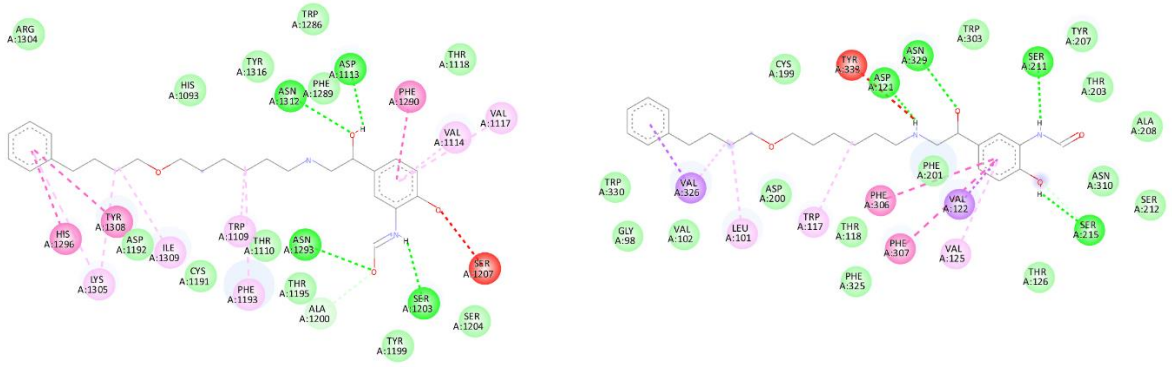

Figure 7. *(a-b) The binding map of analog 2 to β2AR and β1AR.*

3.2.2 Analog 67

Analog 67 comprises formoterol's head group, ethanolamine group, and vilanterol's tail group, with the chlorine atoms shifted and replaced by ethanol (Table 1). To increase π interactions, a

double bond is added to the tail group. When docked to β2AR, hydrogen bonds with Ser203, Asn293, Asn312, and Phe193 were observed, but hydrogen bond with Ser207, which is critical to full β2AR activation, was missing (Fig. 8a). π interactions also occurred between the tail group and Phe194 and His296 (Fig. 8a). Analog 67 only has a statistically significantly more negative ΔG than salmeterol and vilanterol likely due to the incomplete head group interactions (-10.0 kcal/mol, p-value<0.01). When docked to β1AR, analog 67 hydrogen bonded with Ser215, Asp121, Thr126, and Asn329 (Fig. 8b). Though formed more hydrogen bonds than salmeterol and vilanterol, analog 67 engaged in fewer contacts than salmeterol and engaged in weaker types of π interactions. Therefore, analog 67 had a less negative ΔG than all control groups (-8.3 kcal/mol, p-value<0.01).

a **b**

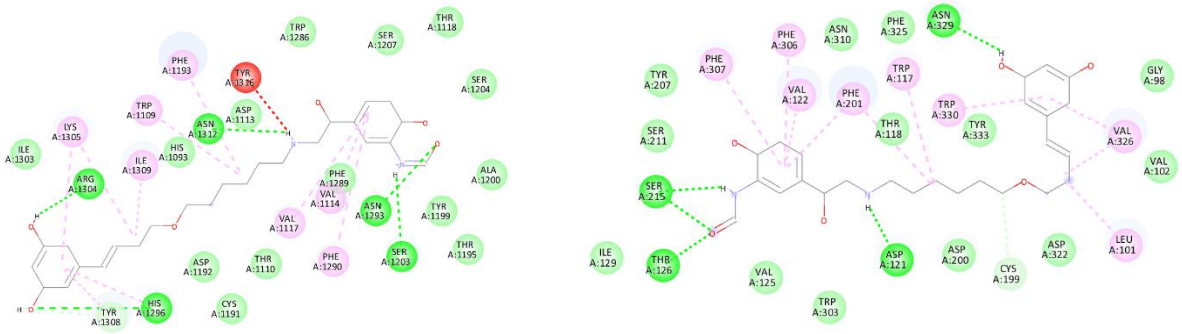

Figure 8. *(a-b) The binding map of analog 67 to β2AR and β1AR.*

3.2.3 Analog 71

Analog 71 comprises formoterol's head group, ethanolamine group, and vilanterol's tail group, except a methyl group is added to increase selectivity, a ring structure is added to increase structural rigidity, and the chlorine atoms are replaced by fluorine atoms to decrease molecular weight (Table 1). When docked to β2AR, hydrogen bonds with Ser203, Asn293, Asn312, Asp113, and Phe193 were observed in addition to halogen bonds with Phe193 and Asp192 (Fig. 9a). Abundance of hydrogen bonds, halogen bonds and other contacts likely led the ΔG to be significantly more negative than any control (-11.2 kcal/mol, p-value<0.01). However, the hydrogen bond network is insufficient to activate β2AR. When docked to β1AR, hydrogen bonds with Ser211 and Asp121, as well as a halogen bond with Gly98, were observed (Fig. 9b). Abundance of contacts likely led the ΔG to be statistically significantly more negative than salmeterol and vilanterol (p-value<0.01) and less negative than formoterol (p-value<0.05).

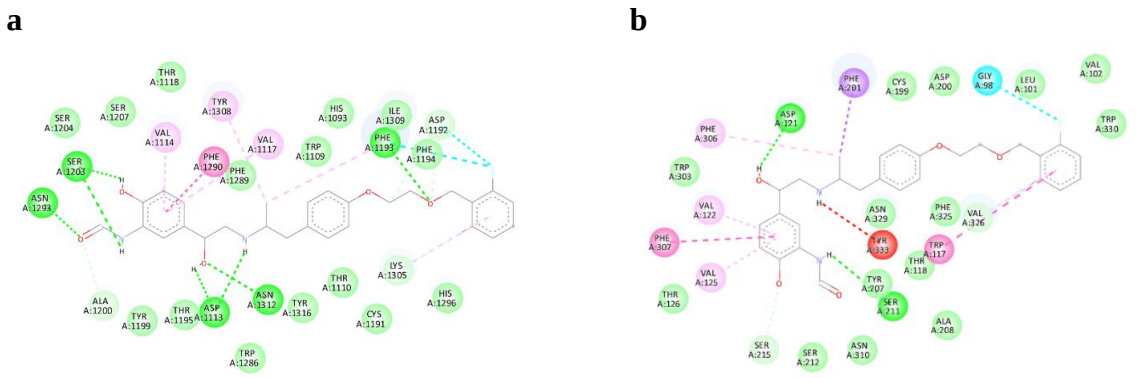

Figure 9. *(a-b) The binding map of analog 71 to β2AR and β1AR.*

3.2.4 Analog 73

Analog 73 is formed by extending the carbon chain of analog 71 (Table 1). When docked to β2AR, hydrogen bonds with Ser203 and Asp113, as well as a halogen bond with Arg304, were observed (Fig. 10a). Abundant contacts likely caused analog 73 to have a more negative ΔG than all controls (p-value<0.05). However, the hydrogen bond network is insufficient to activate β2AR fully. When docked to β1AR, hydrogen bonds with Asn310 and Asp121, as well as halogen bonds with Asp322, Asp200, and Cys199, were observed (Fig. 10b). Reduced number of hydrogen bonds and weaker types of interactions within 4Å of analog 73 likely led to a ΔG that is lower than all controls (-8.7kcal/mol, p-value<0.01).

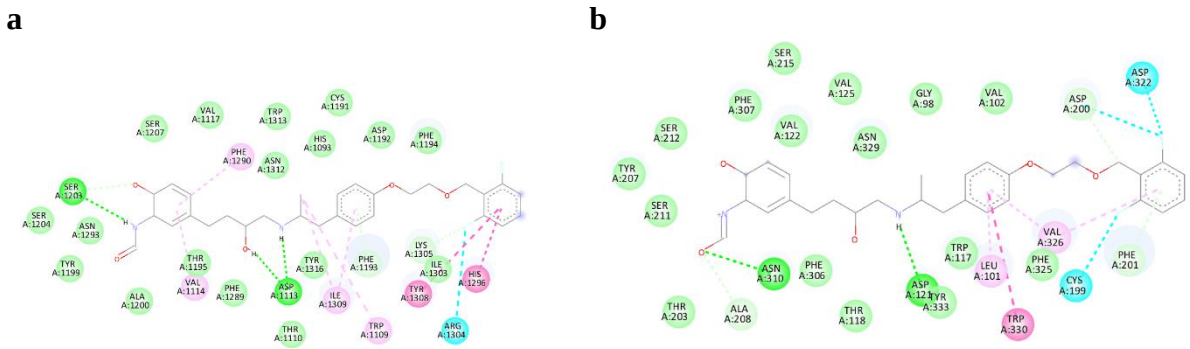

Figure 10. *(a-b) The binding map of analog 73 to β2AR and β1AR.*

3.2.5 Analog 20

Analog 20 comprises formoterol's headgroup, ethanolamine group, and vilanterol's tail group (Table 1). When docked to β2AR, analog 20 formed hydrogen bonds with Ser203, Ser207, Asn293, Asp113, Asn312, and Phe193, the combined set of salmeterol and formoterol's hydrogen bonds necessary to fully activate β2AR (Fig. 11a). Moreover, π interactions were observed between the exosite and the tail group. As such, analog 20 has a more negative ΔG than all controls (-9.9 kcal/mol, p-value<0.05). When docked to β1AR, hydrogen bonds with Ser211, Asn310, and Asp121 were observed (Fig. 11b). Despite the number of hydrogen bonds, analog 20 engaged in three clashes with Phe325, Vall122, and Asn329 with an overlap of 0.683Å,

0.682Å and 0.627Å respectively. Thus, the ΔG of analog20 was less negative than all controls (-8.3 kcal/mol, p-value<0.01).

a **b**

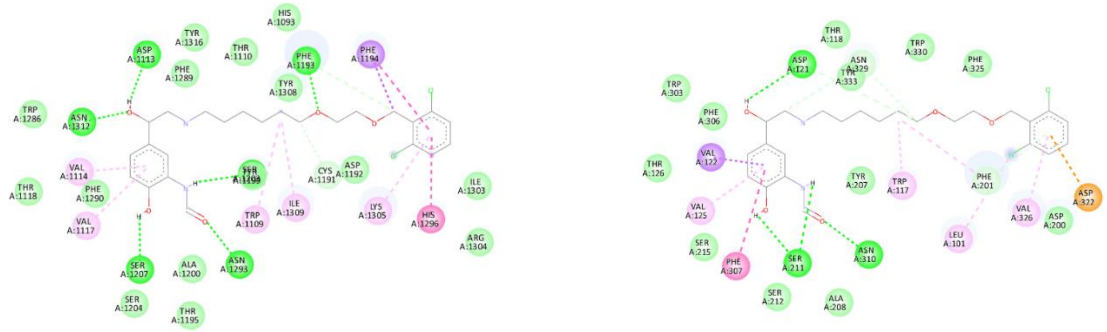

Figure 11. *(a-b) The binding map of analog 20 to β2AR and β1AR.*

5. Conclusion

This *in silico* study combined structural groups of various β2-agonists to create analogs of LABA, leveraging existing known correlations between structural groups, interactions, and physiological effects. Among the 76 novel analogs designed with this method, analog 20 had a ΔG of -9.9 kcal/mol and -8.3 kcal/mol when docked to β2AR and β1AR, which corresponds to a 1.5-fold increase in binding affinity for β2AR compared to the most efficacious LABA and a 4-fold decrease in binding affinity for β1AR compared to the most selective LABA. Moreover, when docked to β2AR, analog 20 formed all hydrogen bonds (Ser203, Ser207, Asn293, Asp113, Asn293, and Phe193) and π interactions necessary for full β2AR activation. Quantitative and qualitative analyses suggest that analog 20 is not only the first analog of LABA to be selective and fully efficacious but also the first to outperform current LABAs in both efficacy and selectivity *in silico*. Biochemical assays should be conducted to confirm these *in silico* results.

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