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

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Introduction

- Asthma is the **most common** chronic disease among children, affecting **262** million patients worldwide and accounting for **450,000** deaths annually.[1]
- Long-acting β2-agonist (LABA) is a frontline asthma treatment, binding to β2 adrenoceptors (β2ARs) on airway smooth muscle cells to induce bronchodilation.
- Unfortunately, current LABAs sacrifice efficacy or selectivity for the other, either increasing risk for cardiac arrest by **2-fold** or **weakening bronchodilation** effects.[2]

Literature Review

- Studies developing analogs (i.e., drug candidates) of LABAs also sacrifice efficacy or selectivity for the other.
- The challenge is that β2AR and β1AR share one of human's most conserved ligand-binding sites: a mere 0.58 Å structural deviation, 92% gene similarity, and only one differing residue in their ligand-binding sites.3
- The popular virtual high throughput screening method is ineffective in discovering simultaneously selective and efficacious analogs.

Year	LABAs	Efficacy	Selectivity (for β2AR over β1AR)
1972	Formoterol^	Full agonist	200-fold[4]
1983	Salmeterol^	Partial agonist	1000-fold[4]
2009	6 analogs+	Inverse agonists	Negligible[5]
2013	Vilanterol^	Partial agonist	1000-fold[6]
2013	6 analogs+	4 full agonists + 2 partial agonists	Negligible[7]

Table 1. *Efficacy and selectivity of approved LABAs and published analogs of LABA.*

^ Approved LABAs
+ Analogs of LABA selected through virtual high throughput screening

Literature Review

Virtual high throughput screening fails to leverage the following known connections between LABAs' structural groups, receptor interactions, and physiological effects:

- Each LABA consists of
  - **A head group**: hydrogen bonds with Ser203 and Ser207 are needed to activate β2AR fully.
  - **An ethanolamine group**: hydrogen bonds with Asn312 and Asp121 are needed to activate β2AR.
  - **A tail group**: π interactions with exosite are needed for selectivity.
- The head group of formoterol accounts for its full agonism and higher efficacy.
- The tail groups of salmeterol and vilanterol account for their higher selectivity.

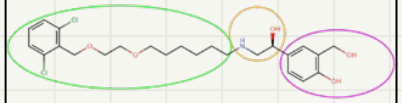
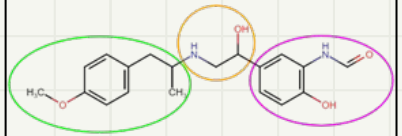
Salmeterol	
Formoterol	
Vilanterol	

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

Purpose

To design the first selective and fully efficacious analog of LABA.

Hypothesis

If structural groups of different current LABAs are combined with consideration of known connections between LABAs' structural groups, receptor interactions and physiological effects,

then the resulting analog will have the efficacy of the most efficacious LABA currently and selectivity of the most selective LABA.^

^Efficacy is shown when the analog has an equal to or more negative change in Gibbs free energy (ΔG) than the most efficacious LABA when docked to β2AR and forms all hydrogen bonds necessary to activate β2AR. Selectivity is shown when the analog has an equal to or less negative ΔG than the most selective LABA when docked to β1AR. This is because more negative ΔG signifies a more energetically favorable binding pose and higher binding affinity. Binding affinity to β2AR should be high and low for β1AR.

Materials

1. A laptop
2. Various open source softwares including Avogadro, AutoDock Vina, Chemaxon MarvinSketch, Discovery Studio Visualizer, SwissADME and UCSF Chimera
3. Files of β2AR (ID: 6MXT), β1AR (ID: 6IBL), salmeterol (ID: K5Y), formoterol (ID: H98) and vilanterol (ID: GW6)

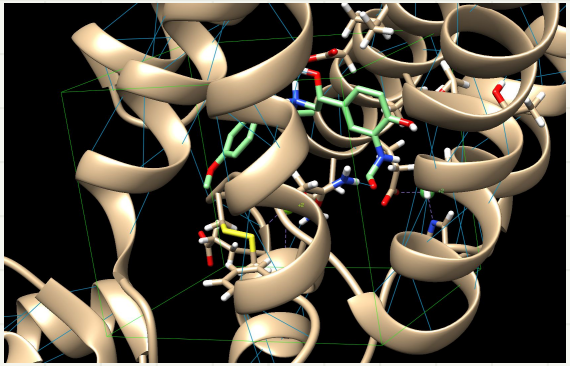


Fig 1. *Molecular Docking Software UCSF Chimera using AutoDock Vina algorithm. The green box is the active site. Protein is in beige color and ligand is in green.*

Note

- **Molecular docking** software was used because it simulates intermolecular interactions between the ligand and the protein, providing more insights for designing better analogs.
- The open-source AutoDock Vina algorithm was used because it allows for flexible ligand and flexible protein side chain docking. Moreover, its scoring system is based on (ΔG), a reliable measure of binding affinity.

General Methods

1. Protein Preparation in UCSF Chimera
2. Molecular Docking of Control Groups (Approved LABAs) to β2AR and β1AR for Five Trials Each in AutoDock Vina
3. 2D and 3D Designing of Analogs using ChemAxon and Avogadro
4. Testing of Analogs for Drug-likeness using SwissADME
5. Molecular Docking of Analogs to β2AR and β1AR for Five Trials Each in AutoDock Vina
6. Qualitative and Quantitative Analysis in UCSF Chimera, Excel, and Discovery Studio Visualizer to Find Insights for Designing Better Analogs.
7. Steps 3-7 were repeated, totaling to 76 novel analogs.

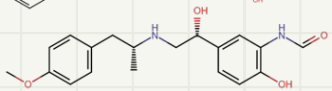
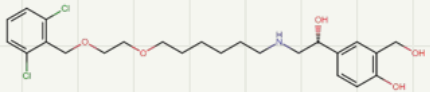
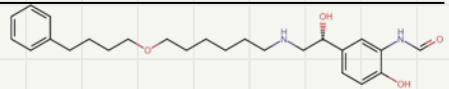
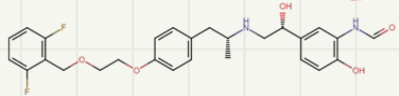
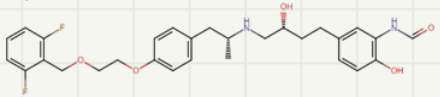
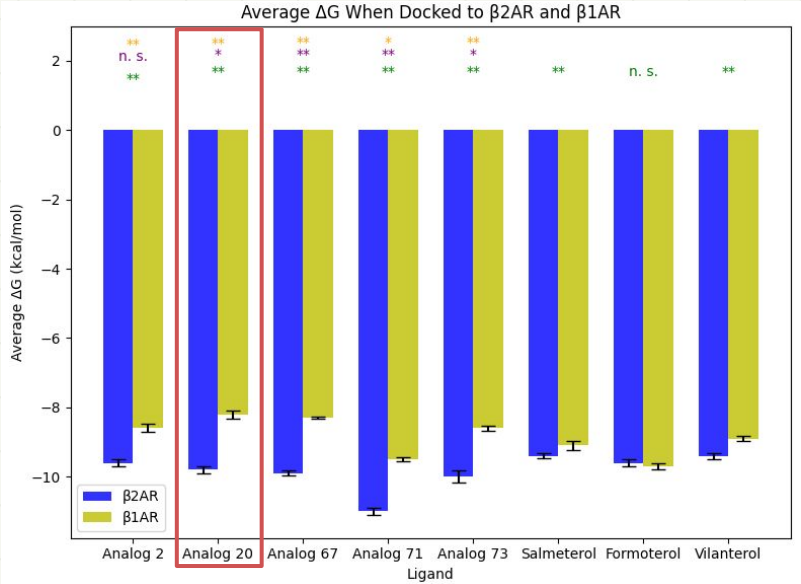
Ligand	Chemical Structures
Salmeterol	
Formoterol	
Vilanterol	
Analog 2	
Analog 20	
Analog 67	
Analog 71	
Analog 73	

Table 3. *Chemical structures of salmeterol, formoterol, vilanterol and some of the analogs*

Results and Quantitative Analysis

Analog 20 had a ΔG of -9.9 kcal/mol and -8.3 kcal/mol when docked to β2AR and β1AR, respectively, which correspond 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.

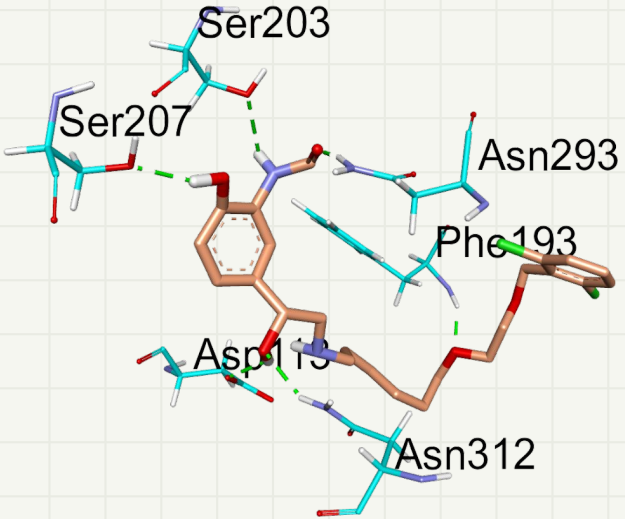


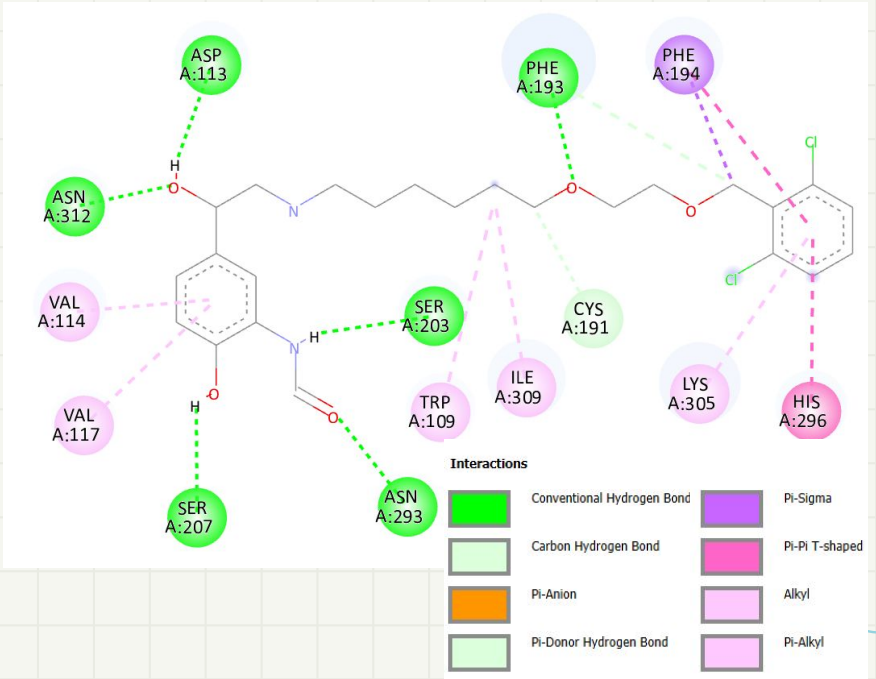
yellow: represents t-test comparing each ligand's ΔG to the control groups for β1AR
purple: represents t-test comparing each ligand's ΔG to the control groups for β2AR
green: represents t-test comparing each ligand's ΔG for β2AR and β1AR
* p-value<0.01 ; ** p-value<0.05 ; n.s. p-value>0.05

Ligand	ΔG in β2AR (kcal/mol)	ΔG in β1AR (kcal/mol)
(R,R)-Formoterol	-9.6	-9.8
(R)-Salmeterol	-9.4	-9.2
(R)-Vilanterol	-9.5	-9.0
Analog 2	-9.8	-8.8
Analog 20	-9.9	**-8.3**
Analog 67	-10	**-8.3**
Analog 71	-11.2	-9.6
Analog 73	-10.2	-8.7

Results and Qualitative Analysis

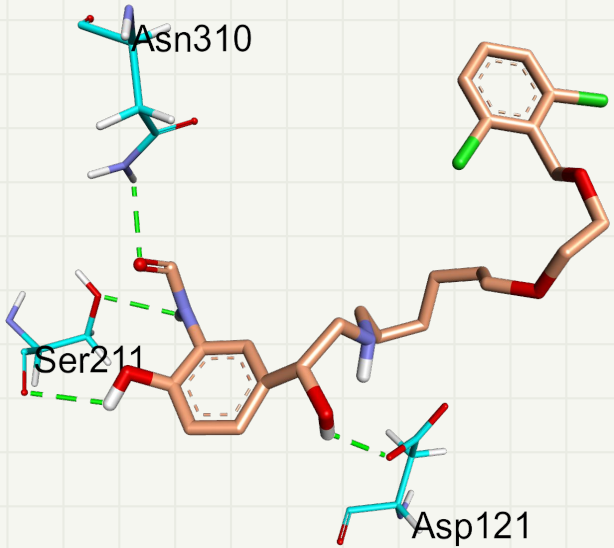
When docked to β2AR, analog 20, which has the head group of formoterol, formed all hydrogen bonds (Ser203, Ser207, Asn293, Asp113, Asn293) necessary for full β2AR activation. It also engaged in π interactions with the exosite, which is critical to selectivity.

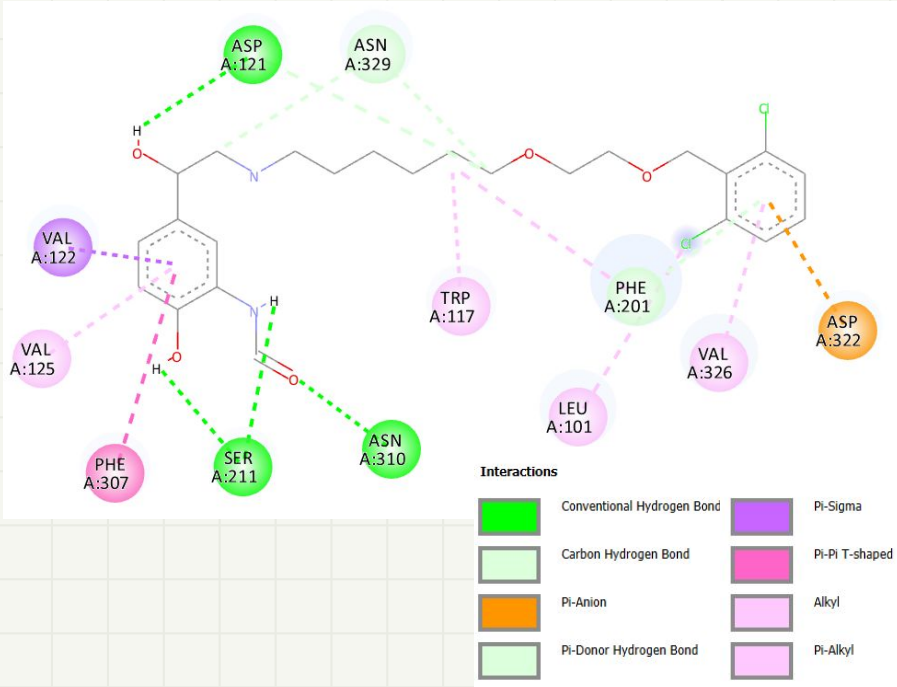




Results and Qualitative Analysis

When docked to β1AR, analog 20, which has the tail group of vilanterol, had fewer of interactions than when it was docked to β2AR, suggesting selectivity.





Conclusion

- Analog 20 had a **1.5-fold** higher binding affinity for β2AR than the most efficacious LABA. It also formed all hydrogen bonds and π interactions necessary for full β2AR activation, suggesting **full efficacy**.
- Analog 20 also had a **4-fold** lower binding affinity for β1AR than the most selective LABA, suggesting **high selectivity**.
- This study successfully designed novel analog 20, which not only is 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*.

Application and Future Work

- Further molecular dynamic simulation and biochemical assays should be conducted to confirm these *in silico* results.
- Given that β2AR and β1AR are among the most conserved pairs of receptors in humans, this study's ability to overcome the challenges of designing a selective and efficacious drug—by leveraging known connections between LABAs' structural group, receptor interactions, and physiological effects—would have significant implications for works attempting to create drugs that target highly conserved receptors.
- This study would also impact works in the newer field of multi-target drugs, especially those dealing with both agonists and antagonists.

References

All photographs and figures by student

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