In silico identification of novel compounds for insomnia treatment

Introduction

Insomnia is a chronic and pervasive sleep disorder that is experiencing an alarming rise in its prevalence in the last decades [1]. This disorder is predominantly characterized by dissatisfaction with sleep duration or quality and difficulties initiating or maintaining sleep, along with substantial distress and impairments of daytime functioning. Currently, clinical treatment for insomnia is cognitive behavioral therapy combined with pharmacological therapy. However, current pharmacological therapy has potential negative effects, such as daytime drowsiness, tolerance, dependance and withdrawal symptoms [2].

The Orexin/OXR system strongly involves in regulating various physiological processes, particularly wakefulness, appetite, and energy balance. Orexin A and B are a pair of neuropeptides that are mainly produced in neurons of the lateral hypothalamus. These peptides bind their cognate G-protein–coupled receptors (GPCRs), orexin receptor type 1 (OX1R) and type 2 (OX2R), which activate different downstream signal pathways, undertaking a variety of physiological functions. OX1R and OX2R are ectopically expressed in many diseases, especially neurological disorders such as depression, addiction and insomnia [3].

Orexin A levels are significantly higher in patients with insomnia disorder than normal sleepers. Moreover, Orexin A levels were detected to have a positive relationship with the course and severity of the sleeping disorder [4]. As Orexin receptors activation promotes wakefulness, antagonists that block orexin receptors would promote sleep urge. OX2R and dual orexin receptor antagonists, but not OX1R antagonists, inhibit wakefulness [5]. These results suggest that narcoleptic effects are mainly mediated by OX2R or a combination of OX1R and OX2R making the OX2R a good protein for developing an antagonist that act as an inhibitor and could treat insomnia.

The objective of this study is to identify potential compounds capable of inhibit OX2R and triggering narcolepsy for insomnia patients. The procedure will focus on increasing the inhibitory potential of approved drugs while trying to minimize their generated side-effects.

Methods

First, a rapid search in ChEMBL database [6] was performed to identify our target protein, Orexin Receptor type 2 (**CHEMBL4792**). Once identified, the ChEMBL RESTful API service [7] was used to search approved drugs for OX2R. Three approved drugs were found where Daridorexant (CHEMBL4297590) show minimal side effects, one of the main problems for insomnia treatment [8].

1. Ligand Based Virtual Screening.

Daridorexant was selected as query molecule for the ligand based virtual screening in order to maintain its low side-effect characteristics for the search. A first screening was performed in SwissSimilarity [9] web platform using the ChEMBL database of active molecules as the compound library and combining 2D and 3D methods for the screening process. Our objective is to discover

molecules that might not be labeled as inhibitors of our target compound but possess the potential to exhibit such properties. For the resulting molecules, MACCS fingerprints (Substructure based) and Morgan fingerprints (Topological based) were calculated using Python's library RDKit [10]. Tanimoto and Dice similarity scores against Daridorexant were computed and all molecules with Tanimoto score below **0.7** for MACCS and **0.25** for Morgan were removed. These thresholds were chosen to maintain a high level of similarity to Daridorexant while ensuring a practical number of molecules. A low threshold for Morgan was set due to the low overall coefficient observed.

2. ADMET Properties Analysis.

ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties are critical factors governing how a drug interacts with an organism. These processes impact drug exposure levels and kinetics, influencing the drug's degradation, processing, and overall activity. To guarantee favorable ADMET properties of the selected molecules an evaluation of the Lipinski's rule of five [11] and the BBB rule was analyzed for every molecule. These rules demands that the compound has between 400 and 500 Da, no more than 10 hydrogen bond acceptors and 5 donors, no acid atoms, a topological polar surface area lower than 140 (related to permeability) and an octanol-water partition coefficient (related with hydrophilicity) lower than 5. The BBB rule was included as orexin receptors are primarily located in the hypothalamus [3], making it necessary for an effective drug to have the capacity to penetrate the blood-brain barrier. Only the molecules that satisfied these 6 properties were selected.

3. Pharmacophore Based Virtual Screening.

Pharmacophoric properties were analyzed to find out which parts of the molecule have biological activity against the target. To build the pharmacophore model the 3 approved drugs against OX2R were used (Daridorexant, Lemborexant and Suvorexant). The rationale behind selecting these drugs lies in the supposition that their shared features are crucial for the inhibition of OX2R. Suvorexant structure is available in PDB linked to OX2R (PDB ID: 6TPJ). A docking procedure using Autodock Vina tool [12] from Chimera [13] was performed for the other two drugs to obtain the conformer that better fit into the protein pocket. To validate this process Suvorexant redocking was executed. These 3 molecules were aligned and individual pharmacophoric features were identified using RDKit [10]. Then, K-means clustering was executed using scikit-learn library [14] to extract the common features and its coordinates. Between the common characteristics of these approved drugs were hydrogen donors and acceptors, aromatic groups and hydrophobic groups. Since selecting all of them was too restrictive, an iterative procedure of searching in Pharmit [15] against a validation dataset and selecting the optimal features was carried out. This dataset included the three approved drugs and the five compounds exhibiting the highest inhibitory potency against OX2R in ChEMBL database. This way a final pharmacophore was obtained consisted in a set of features that match most of the molecules in the validation dataset.

Once the ensemble pharmacophore was obtained, another screening using Pharmit was performed against a database made from our previously validated ADMET filtered compounds.

4. Protein-Ligand Docking

After selecting some drug candidates based on favorable pharmacophoric and chemical properties, a molecular docking was conducted against the target protein (OX2R) to compare binding affinities between the candidates and the approved drugs and estimate their Ki. Autodock Vina tool [12] from Chimera [13] was used to for the docking procedures. To validate the precess and define a suitable docking box, redocking was made with Suvorexant and OX2R (PDB ID: 6TPJ). Therefore, the selected candidates were built in Chimera, their energy were minimized and docking to OX2R was analyzed using the fixed docking box.

Results

Complete code and files used in the analysis can be found in the following Google Drive folder: https://drive.google.com/drive/folders/1bqq_jA6aplJodlvCRzs7RSW77gblupAR?usp=drive_link

Three Orexin Receptor type 2 (CHEMBL4792) inhibitory approved drugs were detected. **Suvorexant** (CHEMBL1083659) with Ki=11.8 nM was the first in approval date, **Lemborexant** (CHEMBL3545367) with Ki = 1.259 nM present the highest affinity and **Daridorexant** (CHEMBL4297590) with Ki = 1.259 nM was recently approved and shows the fewest side-effects [8]. Regarding ADMET properties, the 3 drugs pass the Lipinski's rule of five + BBB rule.

Daridorexant was selected to perform the ligand base virtual screening. **400** molecules were identified using SwissSimilarity tool from which only **82** overcome MACCS and Morgan Tanimoto similarity coefficient thresholds. Among these molecules, **64** of them fulfilled Lipinski's rule of five and BBB rule being molecular weight the most limiting characteristic.

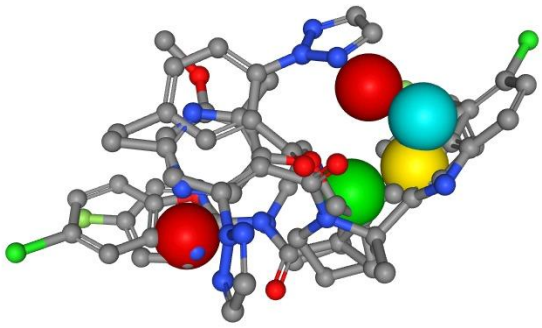

Figure 1: Visual representation of aligned Daridorexant, Lemborexant and Suvorexant and the ensemble features selected for the pharmacophore based virtual screening. Yellow shows hydrophobic groups, blue shows aromatic groups, red show hydrogen acceptor and green shows hydrogen donors.

Before doing the Pharmacophore based virtual screening a docking procedure was performed in which redocking of Suvorexant with OX2R showed 0.2 Å and validated the docking of Suvorexant and Lemborexant. The final ensemble pharmacophoric features consisted in one hydrogen donor, two hydrogen acceptor, one aromatic group and one hydrophobic group as depicted in Figure 1. Pharmacophore based virtual screening against the 64 selected compounds was executed and only **2** molecules with different conformers matched the query pharmacophore. These molecules are **CHEMBL3694264** and **CHEMBL3704928** and their highest scored conformer showed RMSD values of 0.662 Å and 0.664 Å respectively.

The mean results of 5-times docking procedures of the 2 selected molecules and Daridorexant can be observed in Table 1. This docking procedure was validated with the redocking of Suvorexant with OX2R that showed 0.2 Å.

CHEMBL ID	Daridorexant	CHEMBL3694264	CHEMBL3704928
Ki	3.425e-07	1.425e-07	3.183e-07

Table 1: Inhibition constants of Daridorexant and the 2 proposed molecules with the target OX2R.

As shown in Figure 2, both proposed molecules fit perfectly into the main OX2R pocket, in a similar way the approved drugs do. CHEMBL3694264 forms 2 hydrogen bonds with the target, while CHEMBL3704928 lacks any hydrogen bonds.

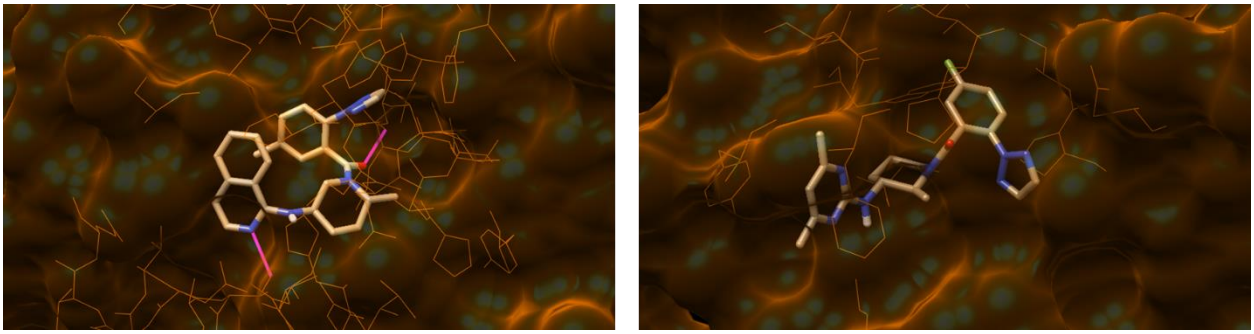

Figure 2: Molecules CHEMBL3694264 and CHEMBL3704928 fitted into the biggest pocket of OX2T. Hydrogen bonds between the molecule and protein OX2R are shown in pink.

Discussion

In this work, an in silico virtual screening was performed with the aim of identifying a novel potential drug candidate for Insomnia. Using a pipeline of different computational tools, we have been able to identify 2 molecules with high potential **CHEMBL3694264** and **CHEMBL3704928**.

For the Ligand based virtual screening, Daridorexant was selected because of its mild side-effect's. The idea was that similar molecules to Daridorexant might show alike side-effects. This characteristic was prioritized over the higher affinity showed by Lemborexant. Choosing Lemborexant as the query molecule might have found molecules with higher affinities and inhibitory power. For the Pharmacophore based virtual screening, highly restrictive characteristics were set seeking to reduce as much as possible the number of molecules in the dataset. More variability can be found by changing the pharmacophore characteristics or increasing the radius of the features.

As seen in Table 1, both proposed molecules show strong affinity to OX2R, with a lower Ki than Daridorexant, meaning improved inhibitory capacity. The improvement is higher for CHEMBL3694264 maybe due to the 2 hydrogen bonds that form with the target as depicted in Figure 2. However, these differences are low making it difficult to assess if these suggested molecules would exhibit significant enhanced efficacy in treating insomnia.

It is totally necessary to analyze in vivo activity to determine whether these proposed molecules are more effective than Daridorexant. It is also essential to assess if the low side-effects characteristic of Daridorexant are maintained for both compounds.

References

[1] E. Aernout *et al.*, "International study of the prevalence and factors associated with insomnia in the general population," *Sleep Med*, vol. 82, pp. 186–192, 2021, doi: https://doi.org/10.1016/j.sleep.2021.03.028.

[2] W. B. Mendelson *et al.*, "The treatment of chronic insomnia: drug indications, chronic use and abuse liability. Summary of a 2001 New Clinical Drug Evaluation Unit Meeting Symposium," *Sleep Med Rev*, vol. 8, no. 1, pp. 7–17, 2004, doi: https://doi.org/10.1016/S1087-0792(03)00042-X.

[3] C. Wang *et al.*, "The Orexin/Receptor System: Molecular Mechanism and Therapeutic Potential for Neurological Diseases," *Front Mol Neurosci*, vol. 11, p. 220, Jun. 2018.

[4] J. Tang *et al.*, "Gender and regional differences in sleep quality and insomnia: a general population-based study in Hunan Province of China," *Sci Rep*, vol. 7, no. 1, p. 43690, 2017.

[5] M. Kalogiannis *et al.*, "Cholinergic modulation of narcoleptic attacks in double orexin receptor knockout mice," *PLoS One*, vol. 6, no. 4, p. e18697, 2011.

[6] A. Gaulton *et al.*, "The ChEMBL database in 2017," *Nucleic Acids Res*, vol. 45, no. D1, pp. D945–D954, 2017.

[7] M. Davies *et al.*, "ChEMBL web services: streamlining access to drug discovery data and utilities," *Nucleic Acids Res*, vol. 43, no. W1, pp. W612–W620, Jan. 2015, doi: 10.1093/nar/gkv352.

[8] A. Markham, "Daridorexant: First Approval," *Drugs*, vol. 82, no. 5, pp. 601–607, Apr. 2022.

[9] V. Zoete, A. Daina, C. Bovigny, and O. Michielin, "SwissSimilarity: a web tool for low to ultra high throughput ligand-based virtual screening." ACS Publications, 2016.

[10] "RDKit: Open-source cheminformatics. https://www.rdkit.org. Accessed 10 Jan 2024.10."

[11] C. A. Lipinski, "Lead- and drug-like compounds: the rule-of-five revolution," *Drug Discov Today Technol*, vol. 1, no. 4, pp. 337–341, Dec. 2004.

[12] O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *J Comput Chem*, vol. 31, no. 2, pp. 455–461, Jan. 2010.

[13] E. F. Pettersen *et al.*, "UCSF Chimera—a visualization system for exploratory research and analysis," *J Comput Chem*, vol. 25, no. 13, pp. 1605–1612, 2004.

[14] O. Kramer and O. Kramer, "Scikit-learn," *Machine learning for evolution strategies*, pp. 45–53, 2016.

[15] J. Sunseri and D. R. Koes, "Pharmit: interactive exploration of chemical space," *Nucleic Acids Res*, vol. 44, no. W1, pp. W442–W448, 2016.