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ABSTRACT

Aim: Insomnia, a cause for sleep deprivation (SD) could result in comorbidities like neurodegenerative diseases (NDDs), as sleep plays an important role in removal of neurotoxic peptides via various mechanisms. Current pharmaceutical therapies are confined to sedation only, instead of considering the whole molecular interlinking pathways that can prevent NDDs and adverse effects also. This research is done to discover a novel drug (DORA) addressing the molecular pathways of sleep recovery and neuroprotection both utilizing the orexin receptors in the brain and enhancing circadian expression and synaptic plasticity. Dual Orexin Receptor Antagonist (DORA) medications are used to maintain sleep and hence stimulate neurowaste clearance mechanism. A synergistic approach combining Artificial Intelligence and Machine Learning (AIML) with computational biology is utilized here, where using a Deep Learning-based predictive model, a list of 12 blood brain barrier (BBB) permeable compounds is extracted from PubChem with 90% similarity to pre-existing DORA (Lemborexant) taking as reference, which all were docked to the Orexin receptors (OxR1 and OxR2) using Swiss Dock, compounds with highest binding affinity were visualized for ligand-protein interactions using BOVIA Discovery Studio Visualizer, ensuring high-precision structural validation. Finally, pharmacokinetics analysis was done by SwissADME to check the efficacy of the drug. Such an AIML integrated approach of drug discovery saves time and cost of wet lab experiments. Furthermore, the drug discovered should be validated for probable action in vivo.

Result: This AI-augmented pipeline identified a novel DORA, (1R,2R)-2-cyclohexa-2,4-dien-1-yl-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-N-pyridin-2-ylcyclopropane-1-carboxamide, having an exceptional binding energy with both the orexin receptors involved in sleep-wake regulation and neuroinflammation, hence serves bidirectionally to treat insomnia along with prevention from NDDs.

Conclusion: A novel DORA is found to treat SD, Insomnia, and, in turn, to prevent associated comorbidities and NDDs like Parkinson's disease (PD), or Alzheimer's disease (AD), etc.

This discovery of novel DORA is done through an in-silico approach, which utilizes AI and a variety of machine learning based techniques and computational algorithms. This method saves a great deal of time, capital, and labor, making it incredibly cost-effective for drug discovery and development; however, the identified new drug can show different activities within the body. Therefore, we advise validating our in-silico findings using in vivo experiments.

1. INTRODUCTION

1.1 Insomnia and its association to NDDs

One's physical, mental, and cognitive abilities are impacted by the physiological need for sleep. Anxiety, stress, and the chance of developing depressive disorder symptoms are all increased by insomnia and sleep deprivation (SD). These consequences are exacerbated by cognitive

deficiencies such as reduced alertness, memory retention, and decision-making[1]. Insomnia is characterized by difficulty in falling sleep or in having an uninterrupted, maintained sleep[2]. Long-term sleep deprivation and insomnia are linked to oxidative stress, neuroinflammation, Lewy body formation, and amyloid beta (Aβ) deposition, all of which are known to raise the risk of dementia and moderate cognitive impairment (MCI) features of neurodegenerative diseases[3]. Insomnia or SD can contribute to occurrence of NDDs by various mechanisms either due to gene dysregulation and epigenetic changes or because of damaged brain physiology after SD or SR including glymphatic system disruption (glial waste removal system), ubiquitin proteasome system (UPS) dysregulation- system for tagging and degradation of misfolded proteins, synaptic dysfunction, microglial priming responsible for neuroinflammation and mitochondrial dysfunction leading to oxidative stress[4]. Hence treating insomnia via drugs targeting through the interlinking pathways can also treat NDDs and not only meant for sedation purpose

1.2 Orexin, Orexin receptors and DORA

Orexin A and orexin B, the two neuropeptides abbreviated as OXA and OXB respectively, are primarily produced by tiny particular cluster of neurons which are located in lateral hypothalamus, they interact with OX receptors 1 (OX1R) and 2 (OX2R) to affect the sleep-wake cycle and stress responses, respectively. By inhibiting the PLC/Ca2+ and NF-kB (nuclear factor-kappa B) pathway, the OX/OXR cascade reactions exhibit neuroprotective and anti-inflammatory actions. While, OX2R binds OXA and OXB equally, OX1R binds OXA firmly rather than OXB by a 1:1 stoichiometric ratio. People who overexpress OXs suffer from sleep deprivation, circadian rhythm abnormalities, MCI linked to insomnia, Parkinson's disease (PD), and Alzheimer's disease (AD) [5].

There were two types of orexin/hypocretin receptor antagonists. These substances are frequently called "Orexants." SORAs which are selective orexin receptor antagonists can either bind to OX1Rs or OX2Rs. While the DORAs that are dual orexin receptor antagonists can interact with both OX1Rs and OX2Rs [6]. Orexin A and B, along with their G-protein coupled receptors (GPCRs), orexin receptor 1 (OX1R) and orexin receptor 2 (OX2R), make up the orexin signaling system [7]. Blocking orexin receptors is a pathophysiological approach to an important medication for insomnia disease because orexins can control sleep by binding to their corresponding receptors [8].

The first DORA to be discovered was almorexant but discontinued to use. The second Orexant or DORA to receive FDA approval on December 20, 2019 was Lemborexant (E2006; DAYVIGOTM) that was developed by Eisai co., Ltd. to treat adults with insomnia. On January 23, 2020, Japan approved it [9]. The use of Lemborexant to treat Alzheimer's disease-related irregular sleep-wake rhythm disorder (ISWRD) is being researched. Lemborexant was authorized for the treatment of adult insomnia in a number of nations, including the US, Canada, Australia, and several Asian nations [10]. Lemborexant is administered orally that is rapidly absorbed but delayed with high-fat meals. It shows ~94% plasma protein binding, a large distribution volume (1970 L), and is primarily metabolized by CYP3A4 into metabolite M10. Clinical trials demonstrated its efficacy for insomnia and circadian rhythm disorders, though adverse effects such as somnolence, fatigue, headaches, and rare complex sleep behaviors (e.g., sleep-walking) were reported. Caution is advised with CYP3A modulators, hepatic impairment, and narcolepsy[11].

1.3 Mechanism of DORA to treat SD and hence NDDs

DORA acts as competitive inhibitor for orexin A and B and acts as antagonist for both GPCRs OX1R and OX2R which promotes wakefulness. Now due to blocking of receptors by DORA, natural ligand that is orexin molecule cannot bind to the receptors and hence the two pathways linking to NDDs got inhibit which are PLC/Ca^{2+} Pathway and NF-κB Pathway [12].

Inhibition of PLC/Ca^{2+} Pathway will lead to no stimulation of Phospholipase C (PLC), hence cleavage of PIP2 in IP3 and DAG [13] will not be possible that will inhibit the release of calcium ions from endoplasmic reticulum, which in turn reduces neuronal excitability causing sleep induction[14].

Dual Orexin Receptor Antagonists (DORAs) regulates inflammatory signals and restore homeostasis in brain that serves as potential approach for treatment of neurodegenerative disorders (NDDs) along with insomnia[15] Orexin-A activates the PI3K/Akt pathway which shows anti-inflammatory effects, due to which the IKK (IκB Kinase) complex suppression takes place. However, this mechanism is seen to be overpowered frequently at phases of chronic disease states [16]. By preventing the phosphorylation and degradation of IκB, the signaling complex is made to exist in a form of nuclear exclusion, the "inhibitor" of NF-κB. Due to this transcription of pro-inflammatory cytokines like TNF-alpha and IL-6 can be prevented [17].

But due to increased orexin activity and prolonged excessive wakening during many NDDs will leads to oxidative stress, ongoing damage, and neuronal over excitation by "over-firing." Use of DORA as therapeutic approach stabilize the sleep-wake cycle, that helps in glymphatic system waste clearance process via CSF to remove the protein aggregates and metabolic/toxic waste that initially cause NF-κB activation. Moreover, DORAs provide a bidirectional action defense, preserving neuronal integrity while decreasing the chronic neuroinflammation typical of Parkinson's and Alzheimer's diseases by blocking the PLC/Ca2+ axis which prevents excitotoxicity induced by calcium, hence prevents inflammatory cascades and also induces sleep simultaneously. DORA by inducing sleep will also make smooth functioning of glymphatic system, UPS system, prevent gene dysregulation and microglial or mitochondrial damage along with synaptic dysfunction and hence in this way too DORA by inducing sleep makes waste clearance possible and prevent the risk for occurrence of NDDs. DORA can be hence used as bidirectional drug for treatment of both insomnia and NDDs.

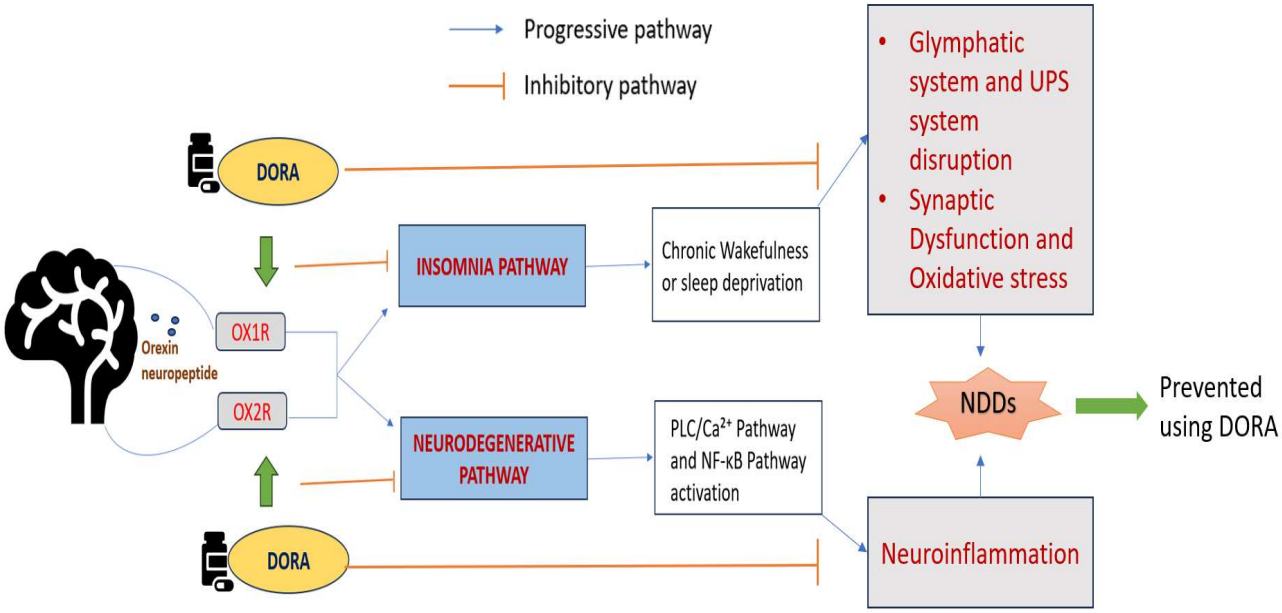


Fig 1. Mechanistic depiction of inhibitory action of DORA on OX1R and OX2R to block the pathway followed by orexin neuropeptide to treat SD and NDDs

Note: In this research, we employed an in-silico drug-discovery workflow- covering tools utilizing machine learning (ML) algorithms to identify novel DORA candidates that may mitigate SD and thereby reduce chances of NDDs like Molecular docking. It is the process of atomic level prediction, to assess the preferred binding affinities and orientations between small binding agents known as ligands and macromolecular structures like receptors known as target [18]. Molecular docking analyzes protein-ligand interactions, finds possible therapeutic candidates, and improves ligand structures to increase binding affinity by modeling the binding process [18].

The discovery of novel DORAs has enormous therapeutic potential, not only for insomnia but also for NDD prophylaxis. In silico drug discovery methods (database mining, molecular docking, and machine-learning aided screening) offer a low-cost method for identifying lead compounds with favorable OxR1/OxR2 binding and pharmacokinetic profiles. Such computational approaches can accelerate early-stage screening, reduce failure rates, and guide subsequent in vitro and in vivo validation.

As a result, the current study aimed to identify compounds that were structurally similar (>90%) to an existing DORA (Lemborexant) and screen them using docking to OxR1/OxR2, interaction analysis, and pharmacokinetic predictions, bridging sleep biology, neurodegeneration, and cheminformatics in a translational context.

2. REVIEW OF LITERATURE

According to an analysis of 27 observational studies, sleeplessness was linked to a 3.78-fold greater risk of one of the NDDs like AD, and in about 15% of cases, effective insomnia treatments could stop the disease's progression [19].

The OX/OXR system is thought to have a significant role in regulating the connection between insomnia and AD [20]. Infusing OX into the hippocampus of Tg2576 mice has been demonstrated to drastically increase interstitial fluid Aβ levels of the brain. Similarly, improved sleep and decreased Aβ levels resulted in an orexin knockout mouse strain. Animal studies suggest that DORAs can improve sleep quality and lower Aβ levels, potentially impacting the progression of Alzheimer's disease [21].

Numerous dual OXR antagonists (DORAs) have shown promise in clinical trials; in 2014, 2019, and 2022, the FDA approved Suvorexant, Lemborexant, and Daridorexant for the treatment of insomnia, respectively [22]. Although they all have certain adverse effects, they are all utilized in accordance with the patient's symptoms. According to the findings of clinical research, a novel medication that targets the OXR system may be able to cure insomnia and the link between sleep deprivation and AD/PD. Treatment with DORAs enhances circadian expression and synaptic plasticity while decreasing Aβ accumulation in the brain.

OX2 receptor activity is primarily associated with sleep-wake cycles and energy balance management, whereas OX1 receptor activation is associated with motivation and reward [23]. The examination of the OX system led to the development of OX receptor antagonists (ORAs), which are used to treat insomnia patients [24]. DORAs, which block both OX1 and OX2 receptors, have shown efficacy in treating insomnia [25]. ORAs are effective inhibitors of OX receptors with anti-insomnia properties [26]. DORAs help alleviate insomnia by reducing GABA and Glutamate release. Their shared sequence matches approximately 64%, but their respective biological activities overlap in some cases, differ in others, providing interesting indications on how one could intervene therapeutically [27].

2.1 Current existing therapies and drugs for insomnia

- **Benzodiazepines (BZDs) –** These works on the GABA receptors, which are positive allosteric modulators (PAM) of the GABA_A receptor that increases the inhibitory neurotransmission [28].
  For Example: Estazolam, Flurazepam, Quazepam, Temazepam, Triazolam.

- **Non-Benzodiazepines (Z- drugs) –** These are also GABA receptor acting drugs but here these drugs are selective for alpha1 subunit of the GABA_A receptor [29].
  For Example: Zolpidem (Ambien), Zopiclone, Eszopiclone (Lunesta), Zaleplon (Sonata).

- **Melatonin Receptor Agonists –** Theses works on the MT1 and MT2 receptors which regulates the circadian rhythm. The drugs used here mimics the natural melatonin and act like it in similar way with the receptors [30].
  For Example: Ramelteon, Tasimelteon, Prolonged-release Melatonin.

- **Dual Orexin Receptor Antagonists (DORA) –** These acts on OX1R and OX2R by blocking them (inhibitory effect). This inhibits the brain waking signals instead of inducing general sedation [31].
  For Example: Lemborexant (Dayvigo), Suvorexant (Belsomra), Daridorexant (Quviviq).

2.2 Limitations of existing therapeutics

- **Cognitive and motor impairment-** Motor incoordination, hangover effects, and daytime sleepiness are some adverse effects of BDZs and Z drugs [32].
- **Respiratory Suppression**- by reducing upper airway muscle tone and hence can worsen obstructive sleep apnea (OSA) [33].
- **Tolerance and dependance-** Rebound insomnia and anxiety upon withdrawal [34]. This will lead to dependency.
- **Affects sleep architecture-** Reduction of REM and deep sleep (N3 sleep stage) [35].
- **Parasomnias-** Can cause sleep walking, sleep eating, sleep driving kind of things which are dangerous[36] [37].
- **Short half-life issues-** some can make sleep onset but not effective in maintaining sleep like Zaleplon.
- **Sleep paralysis and Hallucinations-** Triggers REM related phenomenon like abnormal dreams or sleep paralysis often seen in DORAs [38].
- **Contraindications-** Highly contraindicated in people with narcolepsy [39].
- **Metabolic Interactions-** metabolized highly by CYP3A4, with certain antibiotics and antifungals
- **Dizziness and nausea-** long term hormonal impacts (prolactin) and shows side effects like nausea and dizziness [40].

DORAs among all the existing therapies are considered to be the most good approach now a days, as possess low risk as compared to others and also preserves sleep architecture but still have some limitations like hallucinations, sleep paralysis and metabolic half life issues. Hence there is an important need of discovering a novel DORA which can overcome all these side effects and limitations mentioned above.

2.3 Lemborexant as reference drug

Lemborexant is taken as the reference here. It shows horseshoe conformation in the binding pocket of receptor OX1R that allows for precise interactions with receptor residues, resulting in high binding affinity and significant antagonistic effects. Lemborexant interacts hydrophobically with important residues in the binding site. These interactions strengthen the binding of Lemborexant to OX1R. Lemborexant has a difluoro group which has hydrophobic interactions, this difluoro substituent plays a critical role in increasing the ligand's binding affinity with the receptor. This explains how hydrophobic alteration in the aromatic ring affects the ligand's affinity for OX1R [41].

Lemborexant relies on intramolecular π-stacking and hydrophobic interactions to maintain binding stability. These findings underscore the importance of OXR interaction and imply that lipophilic hotspots and water-mediated interactions may be applicable to other GPCR targets [42]. This provides useful insights for developing targeted therapies for insomnia-linked AD and PD. It also has potential implications in OXR drug discovery.

Lemborexant has some adverse effects and contraindications in people with narcolepsy and is a controlled substance having potential for abuse and can be misused. Concomitant use with strong CYP3A4 inhibitors is not recommended; Risk of suicidal ideation; Schedule IV controlled substance in the US, with lots of adverse effects like somnolence, fatigue, nightmares, and palpitations. Hence, the given study is to screen a new drug (DORA) by taking Lemborexant as a reference for the prevention of SD and reducing the risk of NDDs like AD, etc.

2.4 Approach for Drug Discovery

Drug discovery takes place in various phases, this process involves main steps of Discovery (in silico/ in vitro), Pre clinical testing (in vivo) and then Clinical trials (on humans) and in last post market surveillance is done. It is a high-stake funnel process having around 10,000 compounds at in silico stage to around 250-300 compounds on preclinical to 2-5 compounds on clinical to final compound after FDA approval.

Phase 1: Discovery phase (in silico)

1- **Target Identification:** A biological molecule either be a protein or a gene which is involved in a disease is identified. To identify these targets, large "omics" datasets (genomics, proteomics) are mined by in silico methods (computational approaches).
2- **Target Validation:** establishing that the target can be "drugged" means that the target can be used for action of drug, to be sure that changing this target can truly stops the illness without destroying the cell, researchers employ computer simulations.
3- **Hit Identification (Virtual Screening):** Computers search databases of millions of compounds (such as PubChem or ZINC) for "hits" via molecular docking or other methods to get the molecules that exhibit in strong binding affinity with the target, instead of conducting physical tests it is done virtually.
4- **Hit to lead optimization:** The "hits" have been refined. AI based tools and molecular modeling systems like SwissADME can predict which substances is pharmacokinetically suitable and which are likely to be hazardous. The best survivors are the lead compounds.[43]

Phase 2: Preclinical Phase

1- **In vitro testing:** The drug is tested in lab on cell cultures in petri plates, to check if in silico conditions are also similar in real biological environment.
2- **In vivo testing:** To study pharmacokinetics and toxicology the drug is administered to animal models (like mice, rats, monkeys and pigs).
3- **IND application:** The developer submits an Investigational New Drug (IND) application to regulatory agencies (such as the FDA) if the application is approved. This is a sign to enter human trials.[44]

Phase 3: Clinical trial- Here the drug is tested for safety and efficacy in humans. This the most time consuming and expensive step of the whole process. Done in 3 phases (Phase I, Phase II, Phase III).[45]

Phase 4: Regulatory review and Post market surveillance- A New Drug Application is filed after Phase III. Regulators examine thousands of pages of data to check if the advantages overcome the hazards. The company must continuously monitor the drug's safety in the general population for the purpose of detecting rare adverse effects, once it is on drugstore shelves to sell [46].

2.4.1 Drug discovery approaches in neurodegenerative diseases

It is very challenging to formulate effective treatments for neurodegenerative diseases, which have led high therapeutic advances. To advance the discipline, it is crucial to overcome these hurdles and make use of contemporary drug discovery techniques. There are various unique challenges for the development of drugs for neurodegenerative diseases because of the critical physiology of brain like BBB and slow progression.

- **Permeability through Blood-Brain Barrier (BBB):** To enable BBB crossing, the CNS drugs should have certain physicochemical properties, like molecular weight of <400-500 Daltons, lipophilicity (logP 1-4), and a small number of hydrogen bond donors less than three ideally. Because of the BBB's selective permeability, almost all the large or big sized molecules and even around 98% of small molecules also cannot enter the central nervous system (CNS) through BBB hence a major challenge in development of medication [47].

- **Pathophysiology of Neurological diseases:** Therapeutic approaches targeting multiple pathways are required because of the complex interconnecting mechanisms and metabolic processes of protein aggregation, mitochondrial failure, neuroinflammation, and other processes. Targeting single pathway is always insufficient for treatment of neurodegenerative diseases and often leads to side effects.

- **Slow development of disease:** Clinical trials need long duration of researches and big size cohorts of patient to get the correct idea of medication in terms of efficacy as the progression of NDDs is relatively slow illnesses. Hence high cost and longer time period becomes challenging in drug development due to this.

- **Different disease expression:** There are different symptoms, mechanism, and level of expression of a disease in each different patient, hence it becomes difficult to select a patient for drug development [48].

- **Less validity from Animal Models:** Preclinical models fails to mimic the actual human metabolism and translational system, hence not give the appropriate results or predictions for the developed drug, therefore they have poor prognostic results for therapeutics [49].

2.4.2 Evolution of drug discovery approaches

In the advancement of drug discovery, approaches have evolved from serendipity to computational approaches from traditional to in silico AIML based approaches.

- **Traditional approach: Natural Products & Serendipity-**
  Whole plants, minerals, or animal products are used in accordance with cultural customs (e.g., willow bark for pain, which contains the forerunner to aspirin) is known as ethnopharmacology (Ancient Times–1800s) [50].

  In the early 1800s modern pharmacy starts extraction of active ingredients from plants and animals. Scientists started separating and isolating pure, natural compounds from plants, such as quinine from cinchona bark and morphine from opium (1806) [51].

  Discovery of penicillin by Alexander Fleming in 1928 is an example of serendipitous discoveries of early 20th-century.

- **The Golden era: Rational Design & Classical Pharmacology-**
  Here, there is manufacturing and synthesis of drugs come into force from not only searching ones but actually building medications in the middle of the 20th century.

  Classical pharmacology is the other name for this. There is library of compounds to test which molecule will give what kind of result with different type of receptors and targets on different cells and tissues of organisms [52].

  Rational drug designing is a approach used here in which the active site of target receptor is discovered where the ligand can attach to give the desired output to treat a disease after lead and target identification like a lock and key model.

  High-Throughput Screening (HTS) is developed in1990s, It uses the library of multiple ligands to get a single best fit or lead compound that hit the best to a single target by use of multiplexing techniques in a single go [53].

- **The Genomic Era: Reverse Pharmacology-**
  Its establishment took after Human Genome Project was finished in 2003.

  Scientist start to look into the disease, and their involved genes or proteins, and then make decisions for the chemical that can interfere with the pathway of diseases to treat them instead of finding compound first and then testing for its effect.

  Gene Therapy begins where a shift to proteins, antibodies, and mRNA took place from chemicals like small molecules that alters the one genetic code and immune system [54].

- **Modern & Future Era: AI, In Silico, and Quantum-**
  The era where computational approaches are used to discover novel drugs at the first step instead of lab-based approaches.

  Artificial Intelligence and Machine Learning (Current state- AIML): AI models can predict the binding of drugs to their target protein that after binding what will be the effect and hoe the structure of target will alter and can even check for multiple targets for a single ligand to get the prediction of side effects also for example AlphaFold an AIML model.

Using AI models, one can discover solely new compound that can fir completely in the active site of targeted receptor to have efficient and desired action to treat disease [55].

Digital twins are used to assimilate toxicity profiles on the virtually represented metabolic system of organism before the actual in vivo trial on animal models or humans [56].

Quantum Computing (The 2026 Frontier): As of 2026, here atom-atom interaction can be studied that hoe drugs atom will interact with the atoms of cells or receptors which will reduce the time for drug discovery because of such precisions. The "electronic structure" problems begin to solve by use of by hybrid AI-Quantum systems which is not possible for general classical computers.

2.4.3 Advantages of In-silico and AIML based drug discovery approach

In silico approach provides a wide range of benefits and is the most advance method of drug discovery which is a really fast, cheaper and labor-saving approach of drug development process as compared to all the others approaches[57] in following manner:

- **Time and cost efficiency:**
  Computational screening of ligands libraries at a single go with various AIML based tools and models reduces a lot of time from years of drug discovery to just days only. Also app. billions of money is needed to make a medicine reachable in market with other approaches but with this the cost also reduced as there is no need of lab chemicals equipment or labor work for physical testing and synthesis of drugs[58], [59], [60].

- **Predictions and Success Rates:**
  Toxicity predictions can be made before actually testing on models or humans, also organ specific toxicity predictions can be made like heart or liver toxic etc. AI enhanced clinical success by very higher rates mainly of Phase I [61].

  ADMET Optimization: Predictions related to drug's absorption, distribution, metabolization, and excretion are made precisely (ADMET), using computational techniques making the identification of best hit or lead compound to be used as drug anf hence only ADMET passed drugs are then undergone for further process ensuring the success rate of drug discovery [62].

- **Strategic Innovations-**
  Drug Repurposing: Computational approaches like AIML can search for new applications of preexisting drugs for treatment of other type of disease and here there is also no need of preclinical or clinical testing as they are already been tested and are safe for administration and intake by humans which is now serving for multiple purposes[63].

  Personalized medicine: AI can now help to analyze genetic makeup and can accordingly design the treatment pipeline and drugs according to the short population size or for the individual specifically according to their immune and genetic system for efficient treatment and hence known as personalized medicine [64].

Target Identification: AI can discover disease targets that were not drugged before or disregarded. This is achieved by analyzing massive datasets from transcriptomics, proteomics, and genomics[65], [66].

- **Ethical and Environmental Impact**
  Decreased Animal Testing, chemical wastage, excessive labor work and natural compounds like plants and minerals make this approach ethically suitable. Also no lab work means no waste production or unwanted damages to environment will also protect environment exploitation. In this way in-silico techniques help drug discovery in sustainable manner that are in line with the 3Rs principle (Replacement, Reduction, and Refinement) and minimize the ethical issues and expenses related to animal research by offering highly accurate human-specific predictions [67], [68].

2.5 Molecular Docking (in drug designing)

Molecular docking is a key step in the computational approach for drug discovery, which makes it possible to check the interactions and binding affinities of drug and receptor affordably with conformational predictions and binding affinity scores. Helps detecting the most suitable three-dimensional complex of two molecules interacting together for drug discovery[69] [70][71]

2.5.1 Core Components of Molecular Docking

There are two essential components necessary for a docking simulation [72], these are:

- **Search Algorithm:** This examines the "conformational space" by trying thousands of ligand orientations and shapes to determine which will best fit into the protein's active site [73].
- **Scoring Function:** This is a mathematical model which ranks the generated poses by binding affinities and stability in nature. By measuring the interaction energy (such as hydrogen bonds and electrostatic forces), it ascertains which configuration is more likely to occur in nature [74].

2.5.2 Key steps in docking process

- **Target Selection and Preparation**: The target protein's three-dimensional structure which is involved in particular disease is first retrieved from the Protein Data Bank (PDB) from uniport RCSB. Then the preparation of target is done by refining the structure by adding hydrogen atoms and removing water molecules.
- **Ligand Selection and Preparation**: ZINC and PubChem databases are used to have a list of potent ligands (therapeutic candidates) and then their preparation is also done and the files of their structure are converted in the forms which are required by the particular type of docking tool you are going to use [75].
- **Docking Execution**: The program made different complexes of ligand and target in several position and assigns binding affinity score to each model along with arranging them from

most stable complex to least stable. Here the ligand is made to bind with the active sites of target, there are multiple docking tools which uses different type of algorithms each.

- **Analysis and Validation**: The score and visual examination of 3D models via Pymol or discovery studio type tools is done to analyze the results [76].

2.5.3 Applications in Drug Discovery

- **Virtual screening (VS):** Screening of thousands of compounds virtually to get the best hit that can bind best with the target which serves as very beneficial in saving time and cost.
- **Lead optimization:** It is the process to improve potency and selectivity of a ligands structure.
- **Drug repurposing:** The process to discover new applications of preexisting drugs for treatment of other diseases.
- **Toxicity Prediction**: The target can be checked if the ligand is able to bind multiple targets also except the desired target only. And hence can predict off site bindings and therefore side effects causing toxicity. So good for toxicity predictions [77].

2.5.4 Commonly used molecular docking software

There are multiple types of docking tools based on their scoring function and different algorithms they use as follows:

- **AutoDock Vina-** AutoDock Vina has replaced original AutoDock 4 and now become the most commonly used docking software a little complex but a better approach for docking [78].
  Strengths: It is faster and more accurate than the previous one as it is multithreaded, but sometimes users found it complicated because of grid maps and groups used here [79].
  Recent Updates: Recently multiple ligand docking can be performed using this tool simultaneously also hydrated docking is added too, which incorporates explicit water molecules in the simulation, this can be done by using version 1.2.0 of AutoDock Vina [80].

- **Glide (Grid-based Ligand Docking with Energetics)-** Glide, is known for its precision and accuracy in proper binding positions and was developed by Schrödinger [81]. Precision Modes: "Extra Precision" (XP) for being safe from false positives results and "Standard Precision" (SP) for speedy screening both are incorporated in this tool which makes it a best tool for precision for best binding position of co-crystallized ligand poses. Hence its success rate is very high then other docking tools [82].

- **GOLD (Genetic Optimization for Ligand Docking)-** GOLD can handle various complexity and is well known for configurations as it provides flexibility in proteins structure for better docking process.

  Flexibility: It allows protein to change conformation by providing flexibility to its side chains shape to change to make a library for ligand
  Scoring: GOLD expresses its result in terms of fitness values, the greater the score of fitness

value the better the interaction is. It is unlike to Vina or Glide, where result is expressed in energy scores which interprets as lower the score higher will be the interaction [83].

- **SwissDock-** The Swiss Institute of Bioinformatics provides service of SwissDock. It is very accessible online, as there is no need of powerful local hardware. It is combined with other technologies, such as SwissADME, Swiss target prediction, to predict the safety and metabolism of drugs simultaneously. It can prepare proteins while docking itself so no need of other mechanism to refine protein molecules earlier also it docks on complete surface so even if active site is not known it search by itself that where there is the strongest binding takes place. Recently it incorporated technology like AutoDock Vina and Attracting Cavities method for speed accuracy respectively [84].

- **FlexX-** It works on the strategy of docking the base fragment first of the ligand and then the remaining ligand with the target molecule. Hence it provides speed while screening large libraries against a single binding site as it saves time because of its strategy of base fragment binding first rather than the whole ligand [85].

2.5.5 Algorithm and working of SwissDock

SwissDock, is a customized web server for docking. 2024 update in SwissDock, has incorporated more reliable algorithms in addition to its original engine (EADock DSS) [86].

Two primary engines for SwissDock core algorithm:.

1. **Attracting Cavities 2.0 (AC)**
   Algorithm formulated by The Swiss Institute of Bioinformatics provide high accuracy or precision.

   Mechanism: It finds possible binding sites on the entire surface of protein by searching energetically active sites on it

   Benefit: It gives better prediction. A little time consuming as takes very long duration in searching the overall surface of protein to get the active domains on protein structure but still accurate and more confidential.

2. **AutoDock Vina 1.2**
   This is known for dependability and speed as industry benchmark.

   Mechanism: It works with advanced Empirical Scoring Function and Iterative Local to get the optimal binding position.

   Benefit: It provides speed, making it perfect for preliminary screenings of several chemicals or ligands.[87]

Step wise step workflow:

The Algorithm for SwissDock is fully automated, its workflow places biology before mathematical operations starts.

1. **Preparation (The "Hidden" Phase)-**
   Prior docking SwissDock solves out any structural issue by ligand or target preparations by itself:

   Ligand Preparation: SMILES or Mol2 files are given as input which are converted into 3D structures and then hydrogen atoms are added, applied partial charges by the help of SwissParam for the ligand preparation.

   Target refinement: Here the retrieved protein structure from uniprot in PDB form are used as input, then for preparation standardizing the protonation states of amino acids, and eliminating water molecules is done to make active site available for binding.

   Sampling (Pose Generation)-
   Thousands of distinct ligand orientations are produced here. Vina uses Lamarckian Genetic Algorithm or stochastic global search to rotates the ligand's flexible bonds. It concentrates on topographical cavities for AC

2. **Scoring (Binding Affinity Calculation)-**
   Result is expressed as Scoring Value (Delta G) for each position that is produced stating the binding affinity. The formula for this incorporates a number of physical forces:

   ΔG{binding} = ΔG {vdw} + ΔG {h-bond} + ΔG {elec} + ΔG {hydrophobic} + ΔG{tors}

   The expected binding is stronger (more binding affinity) when the value is more negative (e.g. -9.0 kcal/mol).

3. **Clustering & Ranking-**
   SwissDock results in multiple outcomes. It uses RMSD (Root Mean Square Deviation) to cluster similar orientations. Ranking of clusters is based on the most advantageous binding energy within each cluster.

   The original EADock and Vina are physics-based use calculations for force fields and thermodynamics while the modern versions and SwissDrugDesign ecosystem are incorporating AI:

- **Scoring Functions**: Vina like enhanced version are using Machine learning models trained on the PDBbind database for real world docking to get best binding scores.

- **Neural Network Scoring**: SwissTargetPrediction, uses Random Forest model of Machine Learning for structural docking based on chemical interactions involved in that disease.

Advantages of Swiss Dock:

1. **Blind docking:** If the binding site for drug on target is unknown then blind docking is useful, blind docking can scan the entire protein surface of Orexin receptors to have binding with ligand chosen.

2. **Consistency**: Here the preparation like removing water molecules and adding hydrogens are done by it automatically that reduces the chances of any human error while docking the novel ligand with the target molecule in place of reference molecule. [83]

3 MATERIALS AND METHODOLOGY

To identify and screen a novel DORA drug that can be used as an alternative or as a replacement with better efficiency, the steps below are followed.

3.1 Exploration of target for drug

Using the Drugbank database (https://go.drugbank.com/), the possible targets for drug Lemborexant were found, where both the targets Hypocretin (HCRTR1) or Orexin receptor type 1 (Ox1R) and Hypocretin receptor type 2 (HCRTR2) or Orexin receptor type 2 (Ox2R), face antagonist action of the drug, opposite to orexin-A (PubMed:26950369) and orexin-B (PubMed:9491897) neuropeptides [88]. Orexin-A binding stimulates a rise in cytoplasmic Ca(2+) levels but not such a response in case of Lemborexant binding. Both the receptor targets were chosen to study the binding affinity status of the new compound as DORA.

3.2 Target structures retrieval

Both the identified orexin receptors, HCRTR1 and HCRTR2, were searched on the Uniprot database [89] (https://www.uniprot.org-link). For in silico approach there is the need of 3D protein structure of these target receptors- Ox1R and Ox2R, which were downloaded using pdb code 4ZJ8 (obtained via X-ray diffraction at a resolution of 2.75Å) and 4S0V (obtained via X-ray diffraction at a resolution of 2.50Å), respectively in .pdb format from the Protein Data Bank (PDB) database [90] (https://www.rcsb.org/-link).

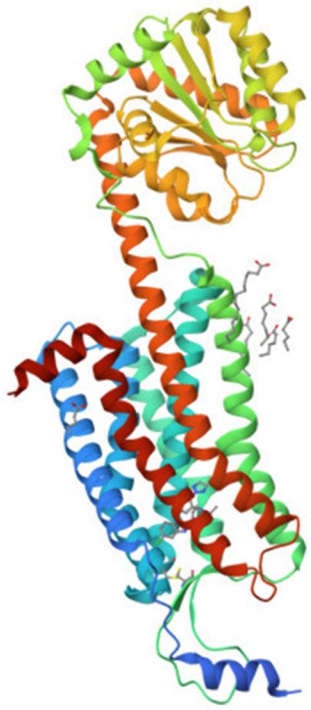


Fig 2. Structure of human orexin receptor(Ox1R) bound to selective and dual antagonist.

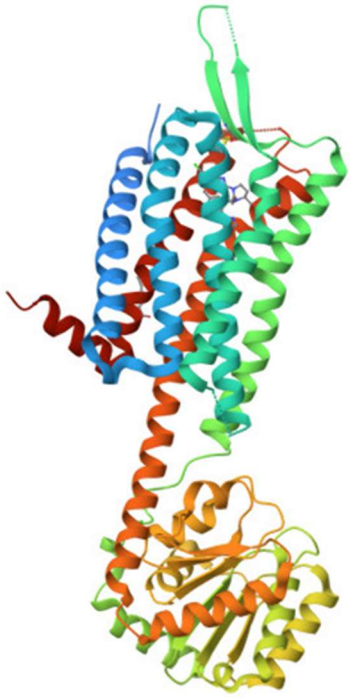


Fig 3. Structure of human orexin receptor (Ox2R) bound to selective and dual antagonist.

3.3 Search for ligands

Similar structure compounds to Lemborexant are searched through a similar compound search to have their analogs that can act as an antagonist on orexin receptors in the PubChem Database [91] (https://pubchem.ncbi.nlm.nih.gov/-link). A list of 657 results was generated at 90% similarity, out of which 12 compounds were selected by filtering out using AI on the basis of molecular weight, rotatable bonds, and heavy atom count within the respective minimum range. These 12 compounds were downloaded as CSV and PNG files for records and as an SDF file in 3D coordinate type to have chemical structures for further processing.

All 12 compounds extracted from PubChem were tested by entering the SMILES format in input for each to find their possible targets via the SwissTargetPrediction tool [92] (https://www.swisstargetprediction.ch/-link).

Lemborexant, as a reference ligand, is used; its 3D structure is also downloaded as an SDF file from the PubChem Database.

3.4 Target and ligand preparation

Using PyMol software, all the water and ligand molecules were removed from the target proteins and saved as a PDB file. For ligand preparation, all the structures of 12 similar compounds and the reference drug, retrieved from PubChem, are saved in .mol2 format using PyMOL.

3.5 Molecular Docking

All 12 drug compounds (ligands) and the reference drug molecule were docked with both the orexin receptors using the Swiss Dock tool, which is a web-based open-access server. The .pdb format of target proteins and the.mol2 format of ligands structure were uploaded on the server [93] (http://www.swissdock.ch/docking-link) and the docking was run one by one of all the ligands and reference drug with each target, taking search box space- center as -5,4,-44 and size as 25,25,25 for Ox1R and search box space- center as 59,7,-28 and size as 20,20,20 for Ox2R. After docking, the resulting zip file is saved, containing an Excel sheet for binding affinities and other details, docked ligands, and target structure files, for further study.

3.6 Protein-ligand complex analysis

Using BIOVIA_DS2025 Client Discovery Studio software, the target structure from the saved zip file after docking, and the best model among the docked ligands are selected to visualize the target-ligand interactions and target-reference interactions in 2D and 3D confirmations. The major focus of observation was the interacting residues and bonds with the reference drug and the selected ligand.

3.7 ADME analysis

All 12 ligand molecules are analyzed using the SwissADME tool [94] (http://www.swissadme.ch/index.php-link) by entering the SMILES format for all. In ADME

analysis absorption, distribution, metabolism, and excretion pharmacokinetics are considered hence it is abbreviated as ADME, respectively, in the acronym ADME. These standards are essential to the development and production of medicinal substances. BBB permeability, the Lipinski rule of five, PAINS, and Brenk are some of the key elements that must be taken into account when developing new drugs.

3.8 Selection of a ligand as a novel drug

The ligand molecule out of those 12, which shows the best ADME analysis, higher binding affinity than the reference drug, no off-target bindings, and similar interacting residues and bonds as the reference, was selected as a novel DORA drug.

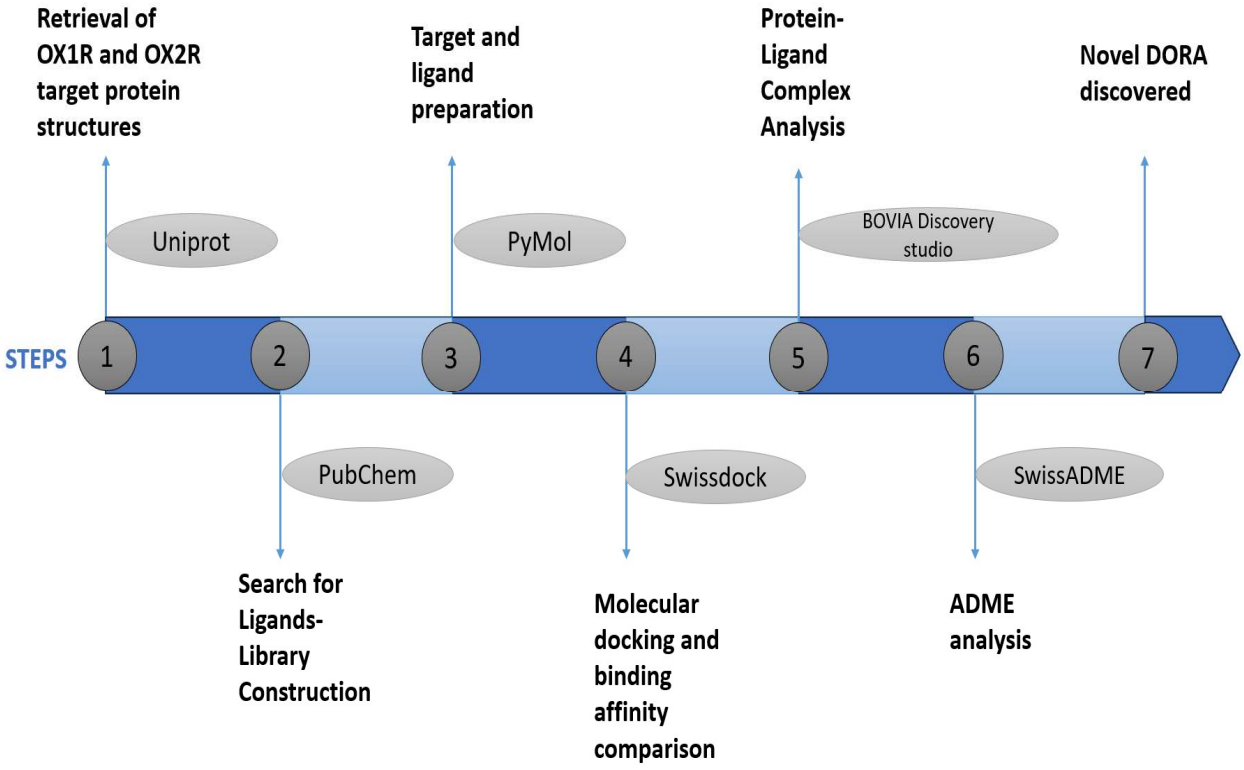


Fig 4. Workflow depicting methodology and tools used for drug discovery (for novel DORA) using molecular docking approach

4 RESULTS AND DISCUSSION

The 12 ligands that are 90% similar to reference (Lemborexant) obtained from PubChem are listed in tabular form (Table I). All these ligands show Ox1R and Ox2R as the most possible targets for them from the results obtained via the SwissTargetPrediction tool, showing very minimal chances of off-site binding, hence less possible side effects.

Fig 5. List of 12 Ligands from PubChem having 90% similarity to reference (Lemborexant)

S.No.	Compound CID	Names
1	**67282745**	(1R,2S)-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-2-(4-fluorophenyl)cyclopropane-1-carboxamide
2	**67473933**	(1R,2S)-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-N,2-diphenylcyclopropane-1-carboxamide
3	**67473934**	(1R,2S)-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-2-phenyl-N-pyridin-2-ylcyclopropane-1-carboxamide
4	**67473935**	(1R,2S)-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-2-phenyl-N-pyridin-3-ylcyclopropane-1-carboxamide
5	**67473936**	(1R,2S)-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-2-phenyl-N-pyridin-4-ylcyclopropane-1-carboxamide
6	**67473951**	(1R,2S)-2-[[(2,4-Dimethyl-5-pyrimidinyl)oxy]methyl]-2-(3-fluorophenyl)cyclopropanecarboxamide
7	**67475236**	(1R,2R)-2-cyclohexa-2,4-dien-1-yl-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-N-pyridin-3-ylcyclopropane-1-carboxamide
8	**67475456**	(1R,2R)-2-cyclohexa-2,4-dien-1-yl-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-N-pyridin-2-ylcyclopropane-1-carboxamide
9	**68083222**	(1R,2R)-2-cyclohexa-2,4-dien-1-yl-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-N-pyridin-4-ylcyclopropane-1-carboxamide
10	**118011994**	(2S)-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-2-(3-fluorophenyl)cyclopropane-1-carboxamide
11	**144350946**	(2S)-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-2-phenyl-N-pyridin-4-ylcyclopropane-1-carboxamide
12	**144350965**	(2S)-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-2-phenyl-N-pyridin-3-ylcyclopropane-1-carboxamide

4.1 Docking results

Docking of all the 12 ligands with both the targets, gave different binding affinities, out of which only single ligand (Ligand 8, Compound CID- 67475456, named as (1R,2R)-2-cyclohexa-2,4-dien-1yl-2-[(2,4-dimethylpyrimidin-5-yl)oxymethyl]-N-pyridin-2-ylcyclopropane-1-carboxamide) achieved the highest binding affinity, even more than the reference with both the orexin receptor when docked at the same site of targets (Fig 5.).

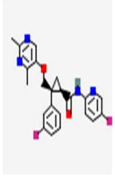
a. *With Ox1R:* Ligand 8 shows binding affinity of -9.52 kcal/mol, higher than the reference, i.e, -8.94

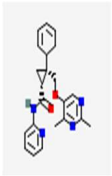
b. *With Ox2R:* Ligand 8 shows binding affinity of -7.04 kcal/mol, higher than the reference, i.e, -6.37.

4.2 Visualization results of docking

The 2D and 3D interactions of ligand 8 and reference were visualized with both the target receptors using BOVIA Discovery Studio. Interacting residues were observed for both the ligand and the reference, as given in Table 1.

Table 1. Binding affinities and interacting residues of reference and selected ligand with each orexin receptor.

Compound	Compound _CID	2D structure	Target	Binding affinity (kcal/mol)	Residues
Reference	56944144 Lemborexant		Ox1R	-8.04	SER315, ASN318, PHE219, ILE314, VAL130, HIS344, GLN179, PRO123, MET183, TYR348
			Ox2R	-6.07	LEU311,

					LEU310, VAL240, ALA314, MET307
Ligand 8	67475456		Ox1R	-9.52	ASN318, PHE219, ILE314, VAL130, HIS344, GLN179, PRO123, GLN126, CYS99, VAL347, GLU204
			Ox2R	-7.04	LEU311, LEU310, VAL240, ALA314, MET307, VAL308, PHE247

The 2D confirmations visualization of reference interaction with Ox1R and Ox2R is shown (in Fig.1 and Fig. 2), and ligand 8 interaction with Ox1R and Ox2R targets are shown (in Fig.3 and Fig.4) respectively.

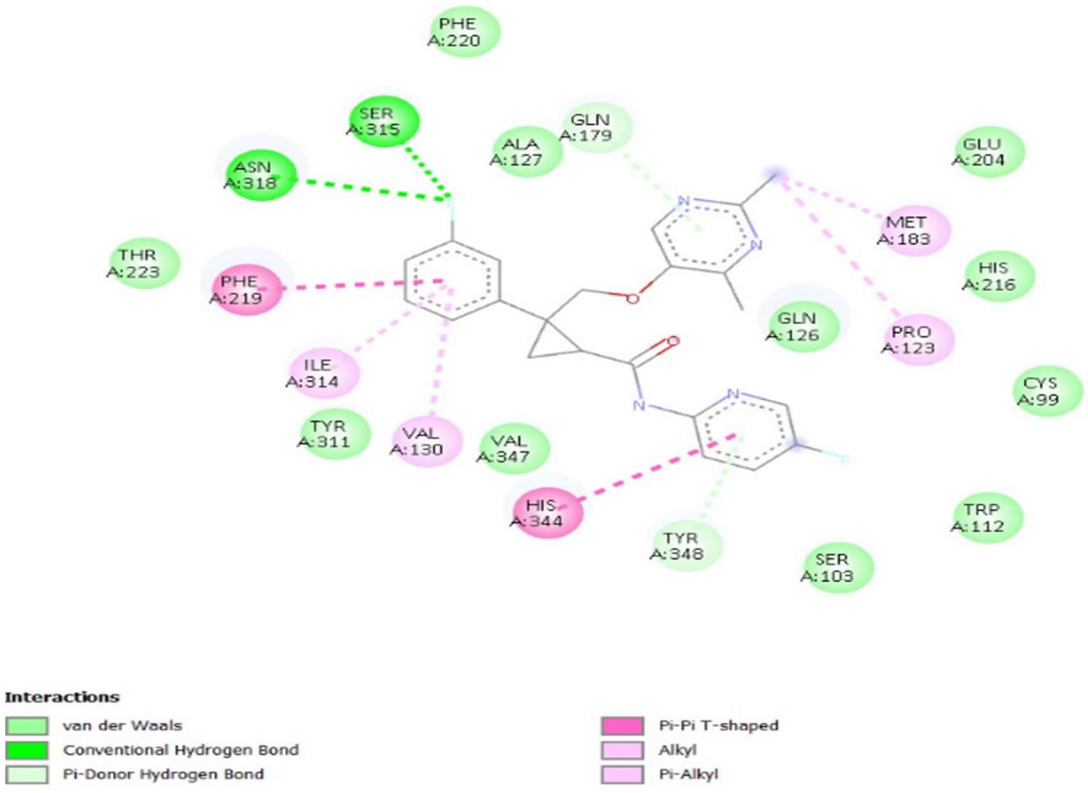


Fig 6. Interaction of reference (Lemborexant) with Ox1R

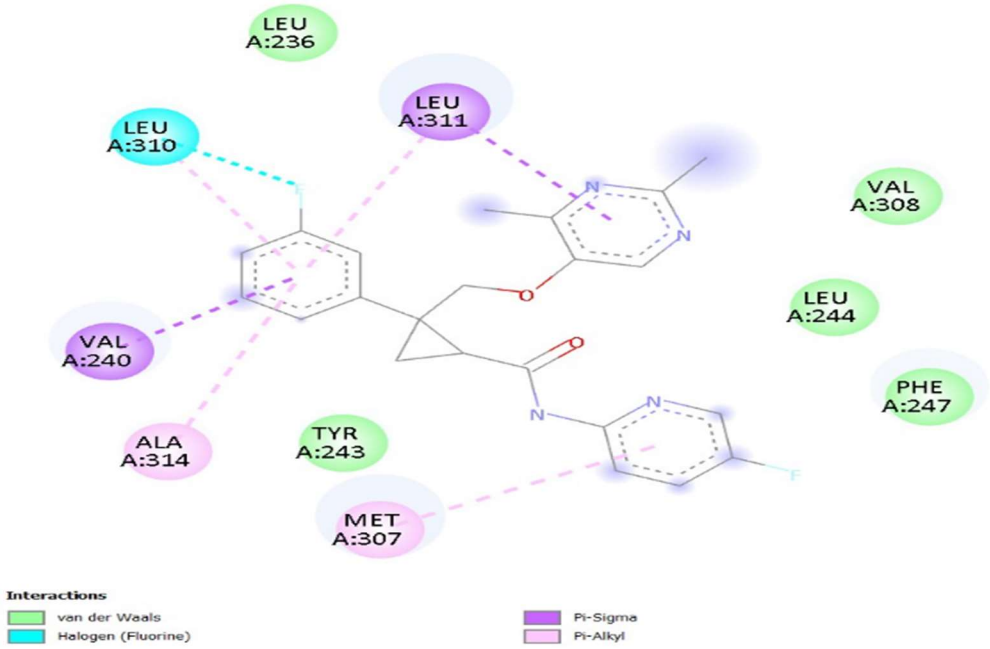


Fig 7. Interaction of reference (Lemborexant) with Ox2R

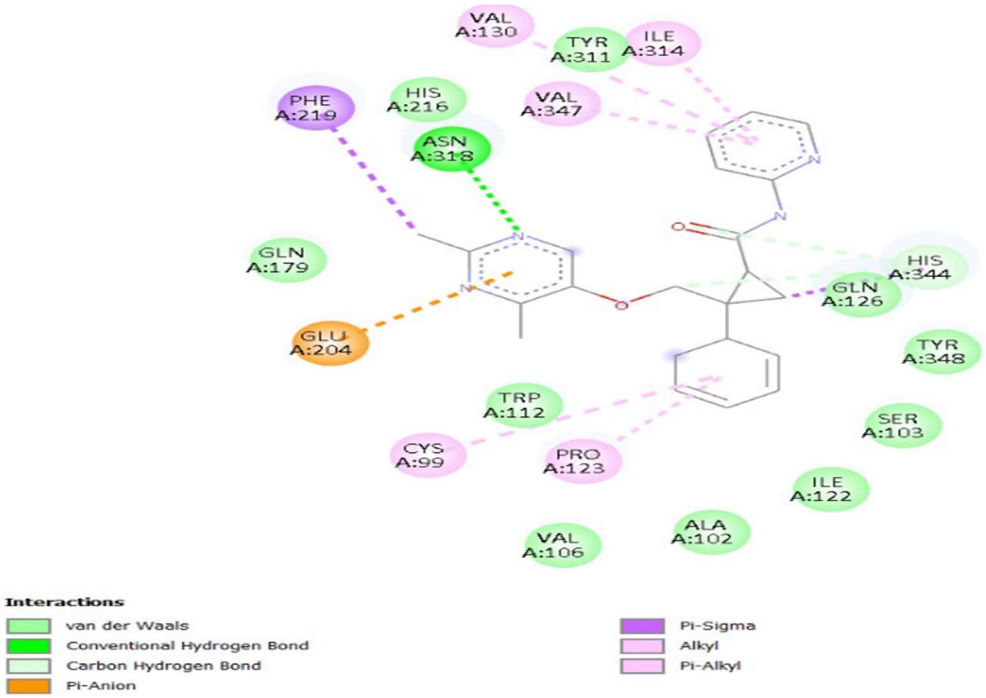


Fig 8. Interaction of ligand 8 with Ox1R

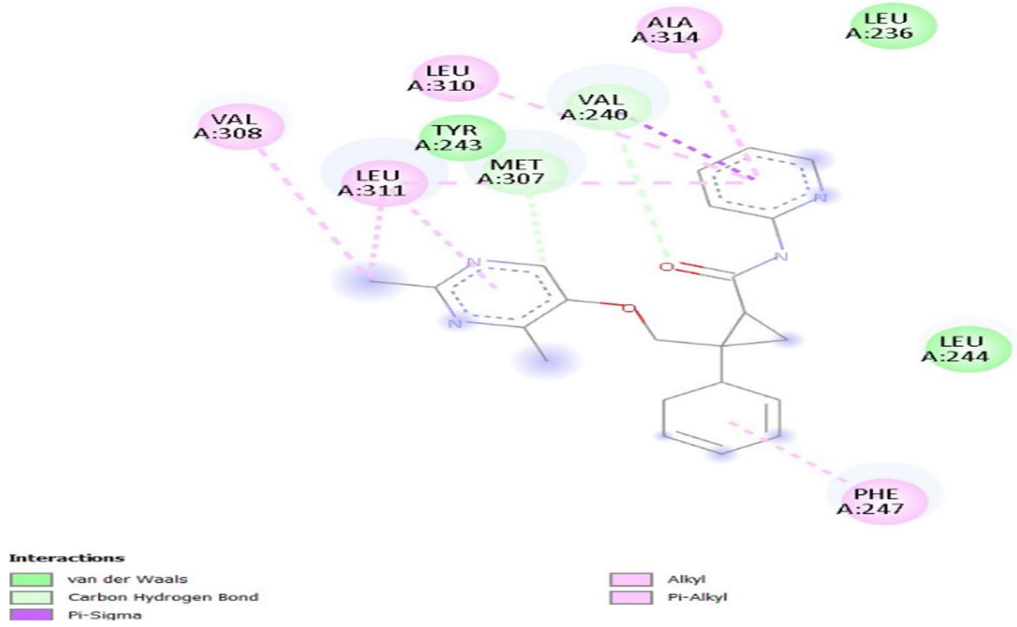


Fig 9. Interaction of ligand 8 with Ox2R

4.3 Detailed ADME Analysis

The resulting SwissADME analysis of the selected ligand 8, depicted this ligand as a favourable drug for insomnia. This compound is BBB permeant with high GI absorptivity, good water solubility, zero Lipinski violation, zero PAINS alerts, and zero Brenks alerts. Also, the TPSA value and bioavailability score, i.e, 77.00 Å² and 0.55, respectively, were found to be similar to the reference drug. Further lipophilicity- consensus log P and skin permeability- logKp values were analyzed, which indicated the effectiveness of the new screened drug as compared to the reference drug by ADME and pharmacokinetics analysis. (Table 2)

Table 2. ADME analysis of the selected ligand 8 and reference (Lemborexant)

Compound	BBB permeable	Lipinski violation	Consensus logP	Gastro-intestinal absorption (GI)	Log Kp (cm/s)
Reference	Yes	No	2.39	High	-6.92
ligand-8	Yes	No	3.02	High	-6.42

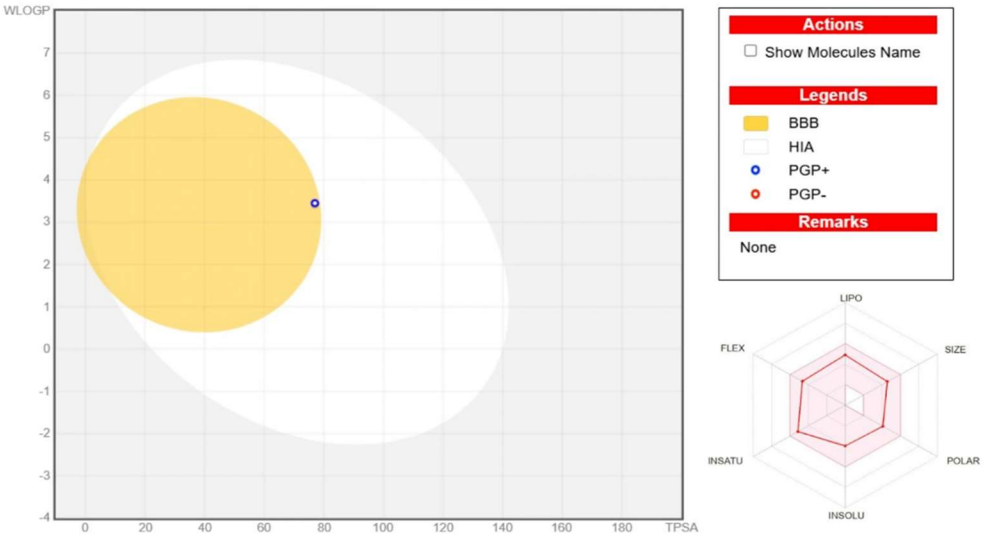


Fig 9. BOILED EGG image for ADME analysis of ligand 8

This in silico research, identified and screened a novel DORA drug through molecular docking interactions between the ligand and orexin receptors with higher binding affinity than the reference drug (Lemborexant), which was further visualized in 2D and 3D confirmations, confirming the common binding residues in the target receptor and also the drug pharmacokinetics through ADME

analysis showed it as an effective substitute of the pre-existing DORA like Lemborexant. Hence, the compound named (1R,2R)-2-cyclohexa-2,4-dien-1yl-2-[(2,4-dimethylpyrimidin-5-yl) oxymethyl]-N-pyridin-2-ylcyclopropane-1-carboxamidecan be used as a drug for insomnia; further in vivo experimentation is needed before approval.

5 CONCLUSION

Sleep deprivation (SD) due to insomnia is a major factor for the progression of neurodegenerative diseases (NDDs) as sleep plays an important role in the removal of neurotoxic metabolites that build up in the sleep deprived brain which can lead to neuroinflammation, oxidative stress, amyloid beta (Aβ) accumulation, alpha-synuclein aggregation and Lewy body pathology which creates the risk for mild to moderate cognitive impairment (MCI) [95] and dementia, hence causes NDDs. Treating SD or insomnia can reduce the occurrence of diseases like Alzheimer's and Parkinson's disease. Orexin receptors (Ox1R and Ox2R), on binding with the neuropeptides orexin A and B, respond in the wake-up state; therefore, inhibition of orexin receptors is required to maintain proper sleep, which can be achieved by using a dual orexin receptor antagonist (DORA) that can treat insomnia and hence in turn enables to prevent the occurrence of NDDs.

In this study, a new compound, ligand 8 i.e., (1R,2R)-2-cyclohexa-2,4-dien-1yl-2-[(2,4-dimethylpyrimidin -5-yl) oxymethyl]-N-pyridin-2-ylcyclopropane-1-carboxamide, is found as a novel DORA to treat SD, Insomnia, and, in turn, to prevent associated comorbidities and NDDs like PD, ALS, HD or AD etc. This is found to be more efficient as compared to other preexisting DORA drugs in terms of permeability through BBB, skin absorptivity, solubility, absorption from gut, no violations, no off-site target binding, having maximum binding affinity with both the orexin receptors. This drug is a type of DORA that can bind to only OX1R and OX2R type of GPCRs, helps in inducing sleep by blocking the action or natural orexin neurotransmitter responsible for reaction of wakefulness, and thus permits smooth functioning of glymphatic and UPS system for clearing and treating waste neurotoxic peptides respectively and also inhibits the PLC/Ca2+ and NFkB pathway which prevents release of several cytokines such as TNF alpha and IL-6 and hence no neuroinflammation takes place. In this way the new discovered DORA can treat insomnia and NDDs both simultaneously by interlinking the molecular pathways and not just only provide sedation or dependency, therefore it is also an advantageous drug over all the other preexisting drugs for insomnia.

This discovery of novel DORA drug is done through an in-silico modern approach of drug discovery to find the lead compound, which utilizes AI and a variety of machine learning based models, tools and techniques along with computational algorithms. Molecular docking via swiss dock is used here which can dock blindly on whole surface of target protein and hence provide better binding with ligand to each site on target and give scoring accordingly if the target site is not known, the binding affinities are then compared out of all multiple results to get the best docked ligand with the target through which we get the resulted discovered novel DORA drug. This method of drug discovery saves a great deal of time, capital, and labor, making it incredibly cost-effective for drug discovery process or development process and hence a better approach for lead identification saving resources and lab expenses; however, the identified new drug can show

different activities within the body. Therefore, we advise validating our in-silico findings using in vivo experiments.