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Research Article

Pharamacohophore Based Lead Optimizatıon and Molecular Docking Analysis for the Detection of Potent Inhibitor against *HCRTR2* Protein

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ABSTRACT

Insomnia is a growing public health problem, as its prevalence is increasing day by day. It is one of the most common sleep disorders among the Asian. Every 1 out of 10 person suffering from sleeplessness have insomnia. Among all sleeping disorders about 6-10% meet the criteria for insomnia. It is highly frequent in women as compared to men. In this research work we focus on identification of potential target based drug in order to provide effective treatment from medicinal plants. In this research study ligand-based pharmacophore approach was used followed by molecular docking and virtual screening. Protox server was use for the prediction of toxicity class. Molecular properties of chemical constituent that possess anti-insomnia properties checked via Swiss ADME, ADMET property prediction tool, and Osiris property explorer tool. Ligand-based pharmacophore of four compound of *Ocimum basilicum* was performed then screened against Zinc, Princeton and Drug bank libraries using ligand scout then compound with higher pharmacophore-fit score with CID NS_013367, NS_005529, NS_007342, NS_011378, and NS_011361. NS_011285 were selected for docking. Molecular docking was performed of desired target *HCRTR2* protein with the screened compounds one by one using Autodockvena and then best pose that show best location orientation angle was selected for further study that exhibit minimum binding energy and docking results were analyzed through Pymol, and discovery studio. Compound with CID number NS_011285 possess best pose and more less binding energy -9.4 as compared to other compounds 2D structure shows the interaction of MET, VAL, LEU, PHE and ARG 2D amino acid with the mutated protein *HCRTR2* and type of interactions such as Alkyl, Pi-Alkyl, Pi-Sigma, and Pi-Sulphur. Finally we identified ligand that possesses best drug likeness properties and potential to inhibit *HCRTR2* activity which is the main culprit of insomnia disorder.

Keywords: Insomnia; HCRT; Docking; Anti-insomnia; Ligand-based pharmacophore modeling; *Ocimum basilicum*

INTRODUCTION

Insomnia is one of the major sleeping disorder characterize by uneasy and discomfort sleep condition. It presents major challenges to public health and is linked with a pronounced failure of activity, physical morbidity, and accidents affect the quality of life, and other psychiatric conditions [1,2]. Insomnia has been reported as a major sleep disorder in Pakistan (42.1% people suffer) [3]. In KPK from statistical analysis it was showed that 11% of the medical students were suffering from insomnia, of which male students were 2.2% and 7.8% were females [4]. People who suffer from insomnia face difficulty in initiating and maintaining a sleep frequent waking during the night and with difficulty getting back to sleep, awaking during night or much earlier than expected. Insomniac patients also complain of associated daytime symptoms such as: tiredness, psychological distress, psychological and behavioral issues or occupational impairments. Approximately 33% to 50% of the insomnia symptoms occur in adult-population; anxiety or weakness (i.e., general insomnia disorder) in 10% to 15%; and serious insomnia disorders in 5% to 10%. Increasing age, female gender, and psychiatric and medical conditions are significant possible causes for insomnia. Insomnia is often associated with substantial psychological, financial and medical consequences, including impaired social activity and quality of life [5]. Insomnia is a common condition of high heritability ranging from 22 to 59 percent. Actigraphy and Polysomnography are used to estimate sleep-wake period [1]. For insomnia assessment and recovery available treatment options includes both non-pharmacological therapies, most commonly cognitive behavioral therapy (CBT) as a first-line insomnia therapy [6] and a range of pharmacological treatments such as benzodiazepines, z-drugs, selective histamine H1 antagonists, melatonin receptor agonists, antipsychotics, anticonvulsants, orexin antagonists, opioids, including non-selective antihistamines [1]. But these drugs lead to several side effects such as drowsiness depression and nausea etc. [7]. As insomnia is sleep disorder link with genetic changes so inhibiting the gene responsible for it can have

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profound effects [8]. Hypocretin are hypothalamic neuropeptides strongly associated with the maintaining of wake/sleep cycle control. The hypothalamic hypocretin system consists of pre-forming hypocretin1 synthesizing neurons hypocretin1 and hypocretin2, which work through two G-coupled receptors; the hypocretin receptors 1 and 2 (HCRT1 and HCRT2) [9]. Receptor genes have been assigned to human chromosomes 1p33 (HCRTR1) and 6cen (*HCRTR2*) and seven coding exons with correlated splice junction positions throughout species were identified for each receptor gene [8]. HCRT cells enhance waking periods and block the onset of sleep. Targeted excitation of ox/*HCRTR2* promotes alertness, so inhibition of *HCRTR2* can provide treatment for insomnia. Medicinal plants are the basic source of medicinal compounds playing significant function in the cure of sleep disorder insomnia [2]. The goal of present study is to demonstrate pharmacophore-based virtual screening in order to predict the novel inhibitor against *HCRTR2* that consolidates wakefulness. Pharmacophore-based molecule libraries were screened against the compounds of medicinal plant that already have been reported in the literature to exhibit anti-insomniac properties. The screened compounds were chosen for further study on the basis of best pharmacophore fit score and were docked with the mutant protein to get best docking result. This computational approach may provide evidence and could help the pharmacist in the design and development of novel inhibitor against *HCRTR2* that block their activity.

RESEARCH METHODOLOGY

Target identification and optimization

Desire target *HCRTR2* was identified by comparative model study of zebra fish through literature review using PubMed which are involved in insomnia. Using uniprot we optimize our target and confirm that HCRT have a role in sleep/wake cycle.

Potential inhibitor identification

As natural plant products have been widely used to treat insomnia worldwide for thousands of years. Potential inhibitors identified on the basis of anti-insomnia compounds included: flavonoids, alkaloids, steroids, saponins, terpenoids, and quinoids (Edewor-Kuponiyi, 2013) NIZBO is the local name of plant its botanical name is *Ocimum basilicum* and belongs to the family Lamiaceae and found as a herb in the area of Bahawalnagar Pakistan [10].

Toxicity prediction

Protox server was used for toxicity prediction then four compounds were taken from *Ocimum basilicum* that lie in the class 5 and 6 as potential inhibitor for insomnia.

Molecular properties prediction

Online tool Osiris Property Explorer was used for molecular properties prediction of *Ocimum basilicum* such as to estimate the possible tumorigenic, reproductive, mutagenic risk, irritant risk and to calculate the drug-like properties.

Retrieval of target protein and ligand compounds

Effective ligand compounds of the plant such as Cineole with Pubchem CID number 2758, cadinol with CID no 6428423, geraniol with CID no 637566 and catechin with 9064, were identified and retrieved from the Pubchem database structural conformations were applied to all the downloaded compound and3D structure of *HCRTR2* protein that is involved in insomnia were downloaded from PDB database.

Generation of pharmacophore

Ligand-based merged feature pharmacophore was generated for all the 4 Compounds to classify the combine attributes of all the ligand compounds in ligand scout software.

Screening

Screening of these pharmacophore compounds were performed against Zinc, Princeton and Drug bank library and 3290compounds were screened out45785 compounds and the top five compounds were selected for molecular docking that exhibit high pharmacophore score.

ADMET properties prediction

ADMET properties and drug likeness properties of all the ligand compounds were checked through ADMET properties prediction tool, PKCSM and Swiss ADME in order to analyze their harmful effect on various organs of living organisms.

Ligand and protein preparation

Ligand compound were prepared for effective docking in the Pymol and discovery studio software while protein was prepared for docking by eliminating the non- standard residues, water molecules, and by adding polar hydrogen atom and kolman charges in Autodockvena.

Molecular docking of hit compounds

Top 5 ligand compounds that possess high pharmacophore score were docked into the *HCRTR2* protein with the help of Autodockvena.

Docking analysis

9 best poses of all hit compounds were retrieved which were then visualized and analyzed in Pymol, chimera and discovery studio.

RESULTS AND DISCUSSION

The objective of our work is to find out potential inhibitor against insomnia through ligand-based pharmacophore modeling and molecular docking. In this study we found chemical constituent which possess anti-insomnia properties from medicinal plant *Ocimum basilicum* which is found in Pakistan. Through literature review we identified that HCRT gene work as a hub for arousal and have significant role in sleep wake cycle. By comparative model study of zebra fish we identified that HCRT overexpression responsible for arousal. HCRT gene code two proteins HCRT1 and HCRT2 both of these perform their activity via HCRTR1 and *HCRTR2* receptors of GPCR family. As zebra fish have only one receptor *HCRTR2*, HCRT2 activation causes arousal while its inhibition causes sleep. ON the basis of model study we choose *HCRTR2* as a significant target that have role in insomnia. Initially we identified list of medicinal plants that have anti-insomniac properties then we narrow down our research and find out those that are found in Pakistan and screened those plant. By checking oral toxicity via Protox server finally we selected *Ocimum basilicum* as a medicinal plant whose phytochemicals lie in class 5 and 6 is shown in Table 1. All the information's about this plant is shown in Table 2. Osiris Prop-

erty Explorer tool was used for molecular properties prediction of *Ocimum basilicum* to check whether they can be drug like. Then we select four compounds as a ligand for targeting receptor protein as shown in Table 3. Mutated 3D protein structure was downloaded from PDB database as shown in Figure 1 while ligand compounds were downloaded from pubchem then ligand-based merged feature pharmacophore were preformed of all the four compounds as shown in Figures in 2, 3 and 4 and screened against Princeton library out of 45785 compounds 3290 were screened after screening 5 hit compounds NS_013367, NS_005529, NS_007342, NS_011378, NS_011361, NS_011285 with high pharmacophore score as shown in Table 4 were chosen for molecular docking. Canonical smile format of these hits were submitted in to online server ADMET property prediction, PKCSM and swissADME in order to predict the pharmacokinetics and drug-likeness properties of these hit compounds that can be used as a drug against human being. These properties includes Intestinal absorption (human), Water solubility, Caco2 permeability, BBB permeability, Hepatotoxicity, AMES Toxicity, P-glycoprotein, Inhibitor, P-glycoprotein Substrate, further drug likeness and bioavailability score was identified according to lipinski, Ghose, Veber, Egan and Muegge rules. All these rule are applied in order to identify drug likeness of these compounds based on some parameters such as TPSA, Molar refractivity, number of HBD , number of HBA and Liphoplicity all the properties of being a drug-candidate possess in the compound NS_011285 as shown in Table 5 for the purpose of docking ligand was prepared in PDB format from Pymol while protein was prepared for docking after filtration of all non-standard residues and addition of hydrogen and kolman charges protein and hit compounds were docked via Autodockvena and the 9 best poses of each 5 compounds were obtained (Figures 5-7). All the five compounds possess 9 best poses with the binding affinity range from -8 to -9.5 as shown in Figures 8 and 9 while binding affinity of compound ID NS_011285 is less than other which is -9.4 so finally we select this compound as a potential inhibitor against *HCRTR2* protein whose activation is the main cause of insomnia.

Table 1: Compounds that lie in class 5and 6 and fulfill Lipinski rule of 5.

Chemical Compound	Toxicity class
Cineole	5
Geraniol	5
Cadinol	5
Catechin	6

Table 2: Pharmacological properties of medicinal plants and their phytochemical constituents.

Medicinal Plants	Pharmacological Properties	Part used	Phytochemical constituents	Chemical compound identified
Ocimum Basilium	1. Antidepressent 2. Anticonvulsant 3. Anxiolytic and sedative 4. Enhance memory retention	1. Essential oil 2. Leaves 3. Aerial parts 4. Seeds	Terpenoids, essential oil, polyphenols, tannins and flavonoids	Cineole, geraniol, linalool, cadinol and sabinene, methyl chavicol, beta –carophyllene and neral, quercetin, myricetin, kaempferol, catechin and eugenol

Table 3: Molecular properties of chemical constituent.

Chemical compounds	Mutagenic Risk	Tumorigenic Risk	Irritant	Reproductive effect	cLogP	Solubility	Mol weight	Drug likeness	Drug-score
Cineole	No	No	No	No	2.109	-2.48	154	-3.214	0.483
Geraniol	No	No	Yes	No	3.485	-1.888	154	-3.569	0.272
Cadinol	No	No	No	No	3.534	-3.291	222	-4.236	0.417
Catechin	No	No	No	No	1.508	-1.736	290	1.921	0.871

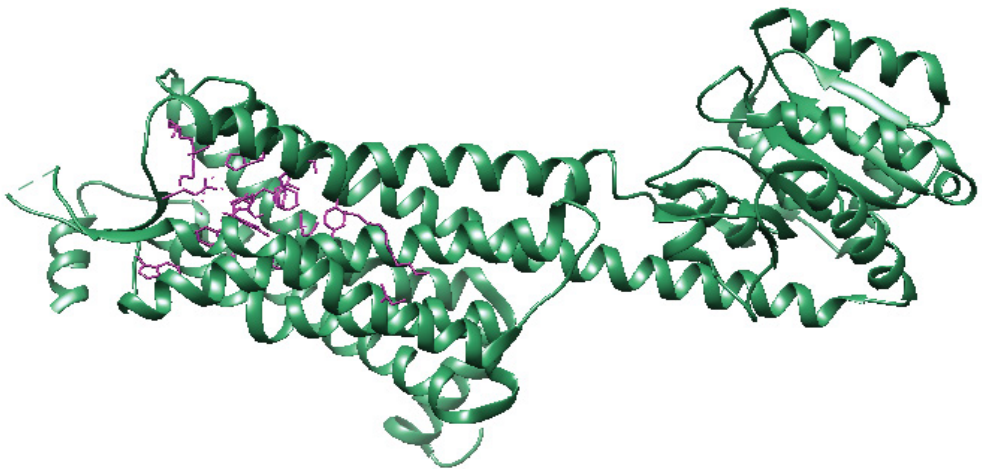

Figure 1: Crystal structure of mutated *HCRTR2* protein.

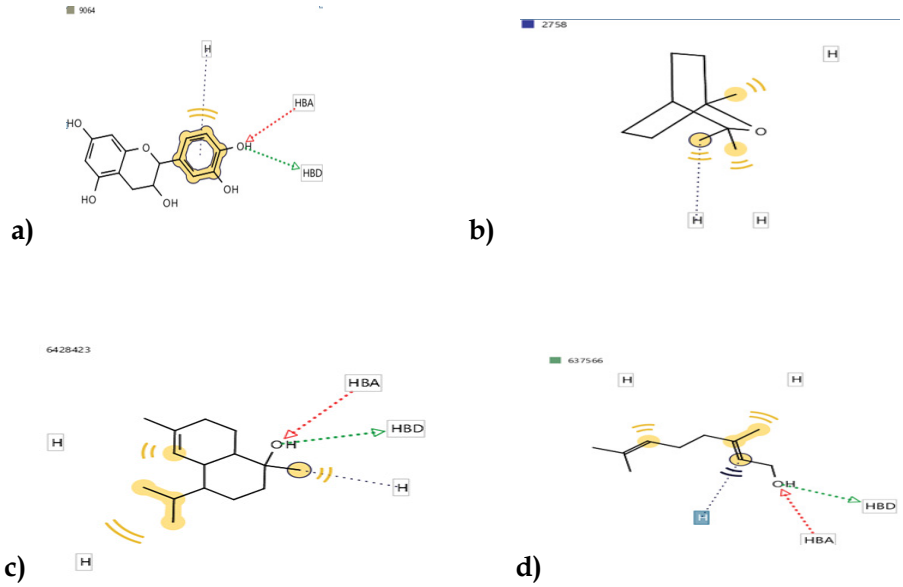

Figure 2: Training datasets for pharmacophore modeling.

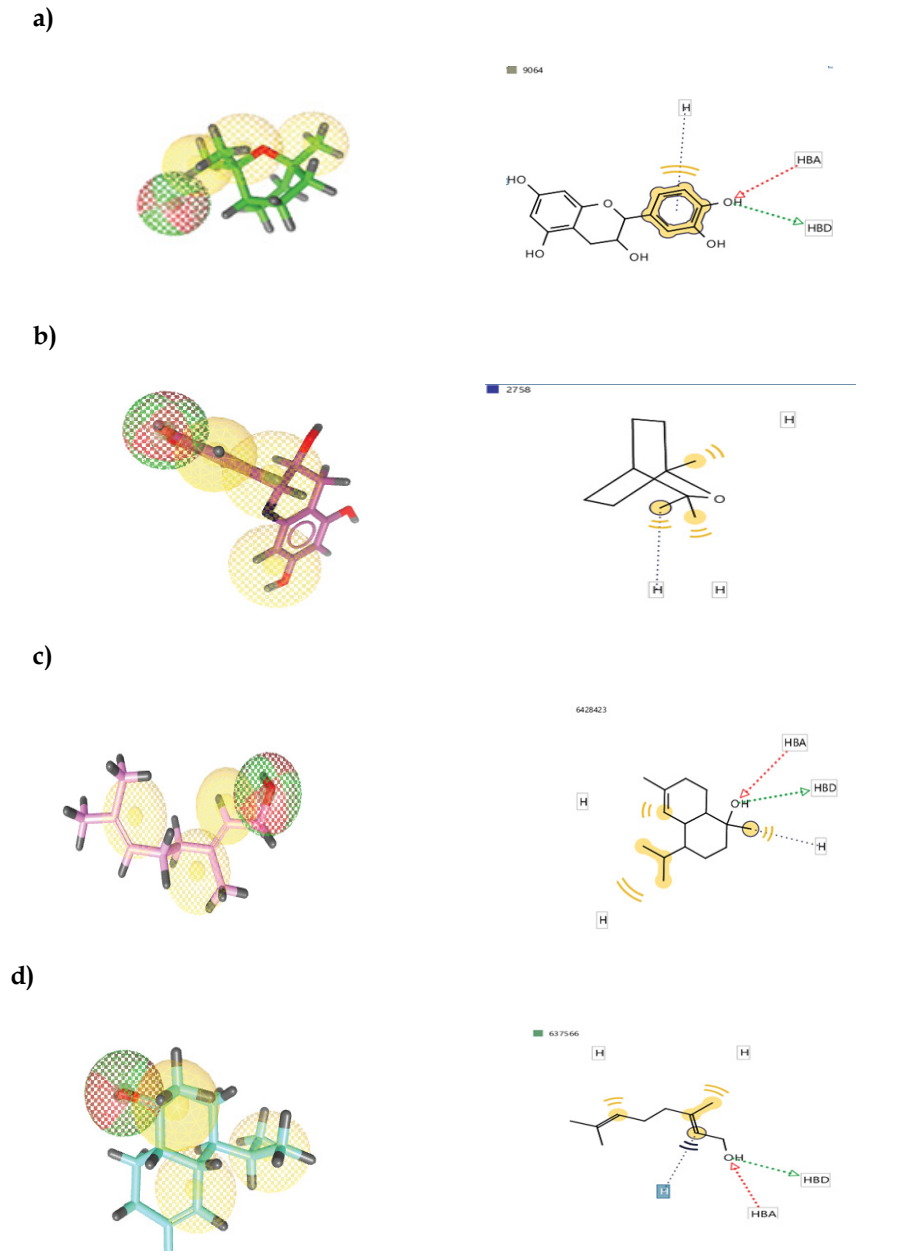

Figure 3: Shows the pharmacophore of all the training datasets. Merged feature pharmacophore was performed in order to find merged feature between all these four compounds such as hydrogen bond donor, hydrogen bond acceptor and hydrophobic bond.

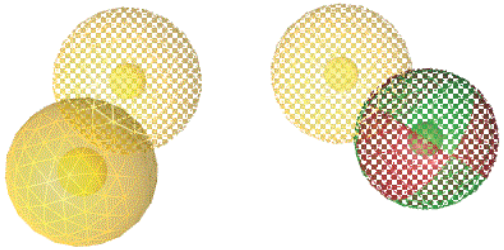

Figure 4: Shows merged feature pharmacophore developed using ligand scout. Pharmacophore feature included Hydrogen bond Acceptors (Red spheres) and Aromatic ring (Yellow Sphere), and hydrogen donor (green sphere).

Table 4: Shows the list of 6 compounds with docking scores and high pharmacophore fit score in hit library.

Compound ID	Compound Name	Pharmacophore fit Score	Docking Score
NS_013367	2-[(diethylamino)methyl]-6-[1-hydroxy-2-(3-methoxyphenoxy)ethyl] phenol hydrate	61.08	-8.0
NS_005529	8-((diethylamino)methyl)-3-(2-methoxyphenoxy)-7H-chromen-4-ol hydrate	61.07	-8.3
NS_007342	7-((diethylamino)methyl)-2-(2,4,5-trimethoxybenzyl)benzofuran-3,6-diol	61.06	-7.6
NS_011378	[({[2-({[1,1'-biphenyl]-4-yl}methyl)-3-hydroxy-1-benzofuran-7-yl]methyl}(ethyl)amino)methyl]oxidanediium; methane	61.05	-8.9
NS_011361	7-[(ethylamino)methyl]-2-[(1-methyl-1H-indol-3-yl)methyl]-1-benzofuran-3-ol; ethanol	61.05	-9.1
NS_011285	[({[2-({[1,1'-biphenyl]-4-yl}methyl)-3-hydroxy-1-benzofuran-7-yl]methyl}(ethyl)amino)methyl]oxidanediium; methane	61.05	-9.5

Table 5: Pharmacokinetic properties of compound NS_011285.

Absorption						
Water solubility	$CaCO_2$ permeability	Intestinal absorption (human)	Skin Permeability	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor
-4.041	1.209	98.796	-3.751	Yes	Yes	No
Distribution						
VDss (human)			Fraction unbound (human)		BBB permeability	CNS permeability
1.32			0.254		0.257	-2.732
Metabolism						
CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitior	CYP2C19 inhibitior	CYP2C9 inhibitior	CYP2D6 inhibitior	CYP3A4 inhibitior
No	yes	No	No	No	No	No
Excretion						
Total Clearance					Renal OCT2 substrate	
1.021					Yes	
Toxicity						
AMES toxicity	Max. tolerated dose (human)	hERG I inhibitor	hERG II inhibitor	Oral Rat Acute Toxicity (LD50)	Oral Rat Chronic Toxicity (LOAEL)	Hepatotoxicity	Skin Sensitization	T.Pyriformis toxicity	Minnow toxicity
No	-0.988	No	Yes	2.67	0.879	Yes	No	0.74	2.169
Properties									
Formula	TPSA	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Molar Refractivity		Lipophilicity c Log $_{Po/}$w		
$C_{26}H_{29}N_{O}3$	41.93 Å²	2	4	2	125.78		3.27		

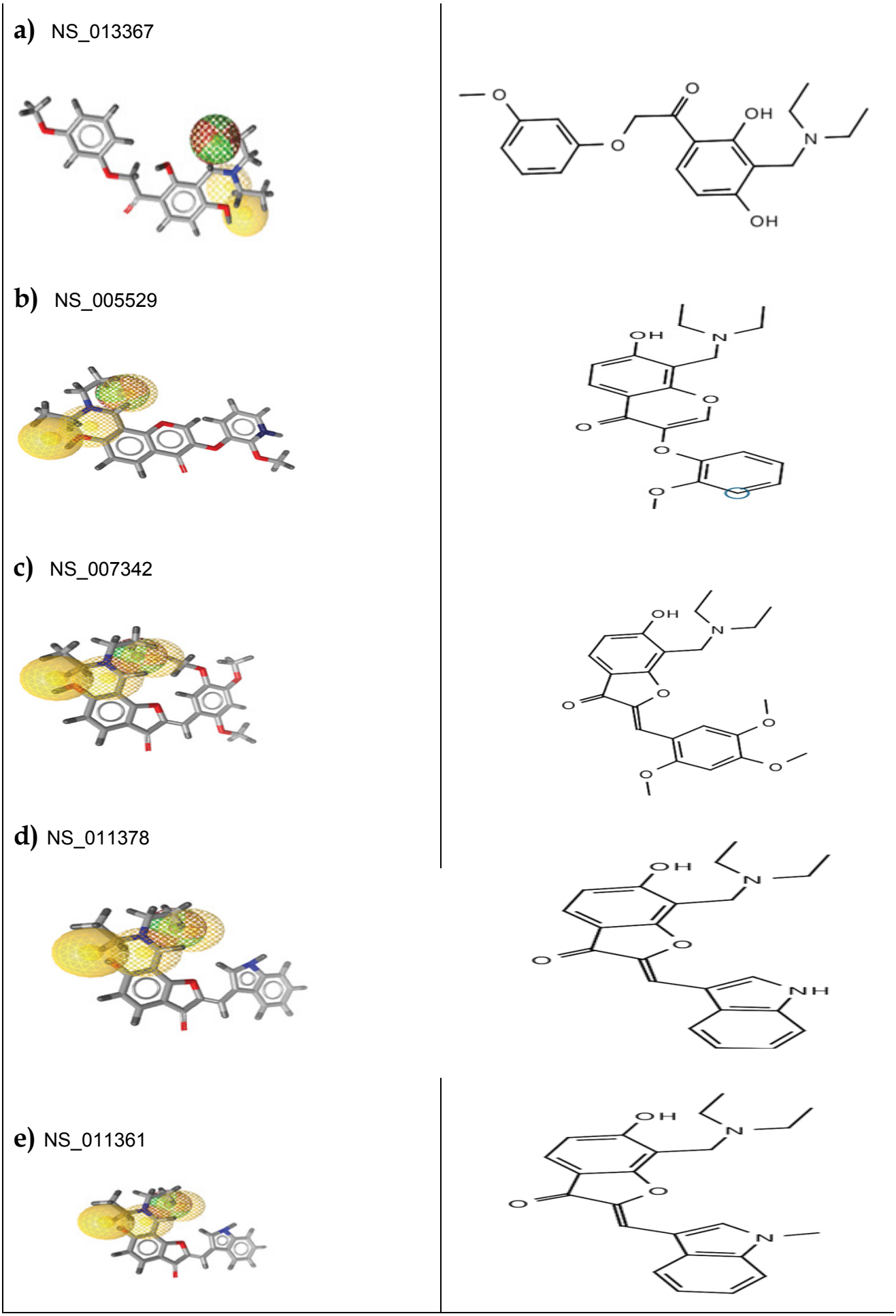

Figure 5: Show the pharmacophore of hit compounds that possess heigh pharmacophore score.

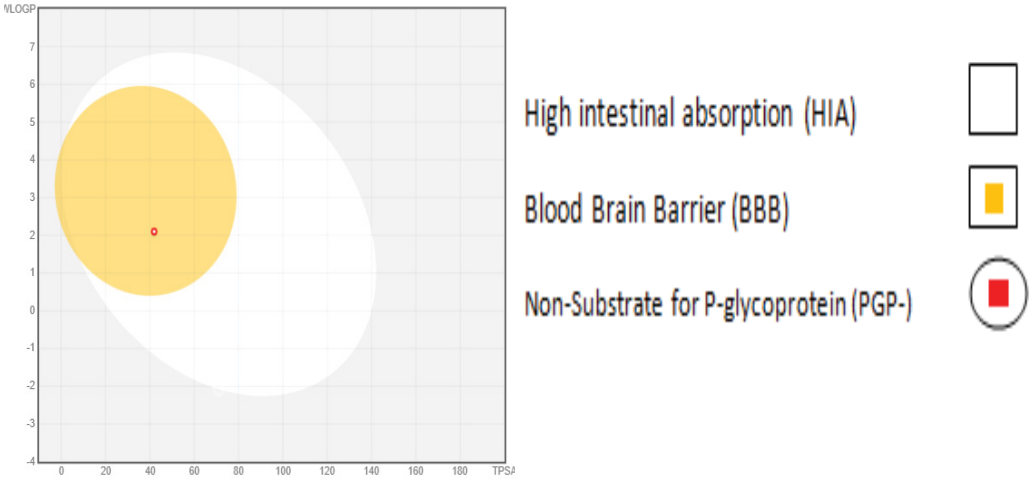

Figure 6: Boiled egg analysis for evaluation of gastrointestinal absorption (HIA) and brain penetration (BBB) with respect to the position of the molecules (water partition coefficient (WlogP) vs. Topological polar surface area (TPSA) of selected lead compounds by SwissADME server.

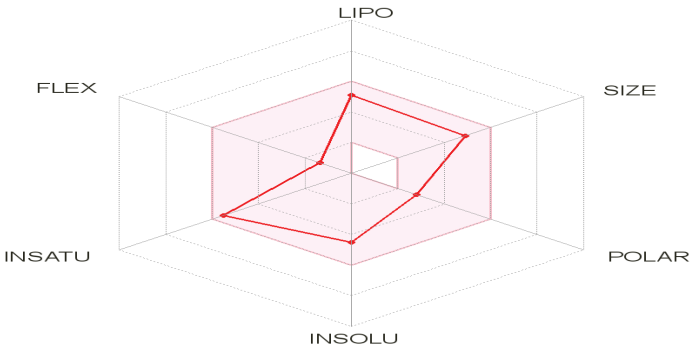

Figure 7: The bioavailability radar accurately estimates the overall drug-likeness of a molecule with id NS_011285.

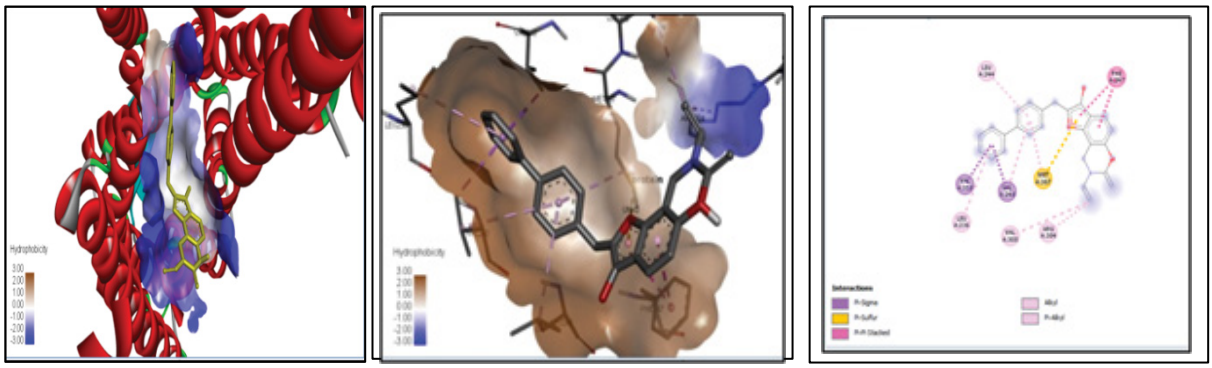

Figure 8: Docking complex of *HCRTR2* protein with compound [({[2-({[1,1'-biphenyl]-4-yl}methyl)-3-hydroxy-1-benzofuran-7 yl]methyl}(ethyl)amino) methyl].

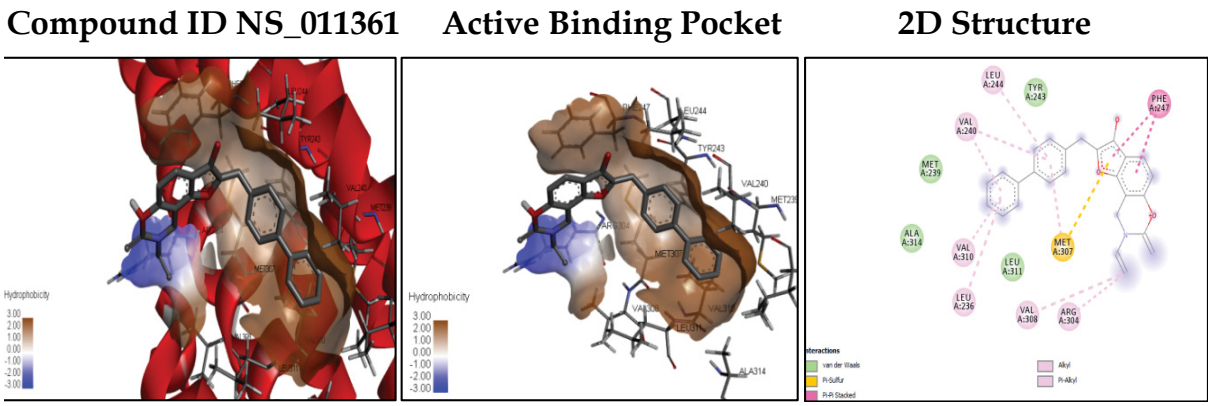

Figure 9: Docked complex of *HCRTR2* protein with compound 7-[(ethylamino) methyl]-2-[(1-methyl-1H-indol-3-yl)methyl]-1-benzofuran-3-ol.

CONCLUSION

Insomnia cause poor sleep and ultimately doomed the person health and personality. This research is conducted in order to identify novel drug targets against insomnia, potential inhibitor against *HCRTR2* protein that will inhibit the activity of *HCRTR2* which is the main culprit of insomnia disease. Literature review and previously conducted study reveals that *HCRTR2* can act as main target against insomnia. To inhibit *HCRTR2* novel inhibitors were identified via ligand based pharmacophore modeling approach and validated by screening and molecular docking that help in the identification of novel and effective drug with lesser side effect and high success ratio. Medicinal *Ocimum basilicum* has anti-insomnia properties. Anti-insomnia properties checked via Swiss ADME, ADMET property prediction tool, and Osiris property explorer tool Ligand-based pharmacophore of four compounds of *Ocimum basilicum* and docking result shows that these phytochemicals of *Ocimum basilicum* act as prominent inhibitors of *HCRTR2* and this inhibition can be effective to design the drugs for the treatment of insomnia. This study suggests more efficacious novel anti-insomnia drug for future study.

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