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In Silico Discovery Of Potential Polycystic Kidney Disease Protein Vasopressin V2 Inhibitors: A Structure-Based Approach Leveraging Tolvaptan-Derived Similarity And Blind Docking

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ABSTRACT

Vasopressin V2 receptor (V2R) Autosomal Dominant Polycystic Kidney Disease (ADPKD) is closely linked with dysregulation of the vasopressin V2 receptor (V2R), and is hence a drug target. A general computational strategy of pharmacophore-guided virtual screening and structure-based molecular docking was utilized in this study to identify potential V2R modulators. The crystal structure of the human vasopressin V2 receptor (PDB ID: 6U1N) was validated and used as the target model. A 3-point pharmacophore model was constructed with Tolvaptan—a well-known V2R antagonist—as a reference, which includes important interaction features such as hydrogen bond donors/acceptors and hydrophobic cavities. A filtered group of compounds from the Zinc Drug database was further assessed with molecular docking using AutoDock Vina and CB-Dock. In the top contenders, CHEBI:130604 scored the highest binding energy with a Vina score of -10.0 kcal/mol, surpassing Tolvaptan (Vina score -9.7 kcal/mol). Other contenders were CHEBI:127778 (-9.1 kcal/mol), CHEBI:128672 (-8.4 kcal/mol), and CHEBI:125791 (-8.4 kcal/mol). Analysis of the V2R pocket showed large cavities, with Pocket C1 (volume: 9069 Å^3) and Pocket C5 (volume: 557 Å^3) being principal binding hotspots. The repeated interaction of high-affinity ligands to these pockets suggests their key role in ligand recognition and receptor modulation.

These findings provide new candidate molecules with more favorable binding profiles than Tolvaptan and offer promising leads to the development of selective V2R antagonists for the therapy of ADPKD.

KEYWORDS: ADPKD; Vasopressin V2 Receptor; Tolvaptan; Pharmacophore Modeling; Molecular Docking; Virtual Screening; Auto Dock Vina; CB-Dock; Binding Affinity; Drug Discovery; CHEBI:130604.

INTRODUCTION

Autosomal Dominant Polycystic Kidney Disease (ADPKD), a frequent inherited disorder primarily as a result of mutation of the PKD1 or PKD2 gene, is characterized by progressive development of fluid-filled cysts in the kidneys.[1-5] Abnormal hyperactivation of cyclic AMP (cAMP) signalling pathways by the Vasopressin V2 Receptor (V2R) is a crucial aetiological mechanism in ADPKD.[6-7]This hyperactivated signalling pathway is central to cyst-lining epithelial cell growth and renal cyst enlargement.[8] Tolvaptan, a V2R antagonist, has been reported to retard progression of ADPKD. Its therapeutic benefit is, however, counterbalanced by potential side effects, e.g., liver damage risk. [9-10]

In the pursuit of safer and perhaps more effective ADPKD therapeutic drugs, drug repurposing is a promising approach.[11] In the present work, we leverage the existing safety and pharmacokinetic profiles of drugs already on the market, offering the potential for a quicker and cheaper route for the discovery of new therapeutics. Computational methods, virtual screening, and molecular docking, in fact, have also been found to be useful for accelerating drug discovery in the context.[12-13] These in silico approaches enable the rapid screening of vast chemical libraries in order to isolate compounds that have a high likelihood of binding to a target biological compound.[14] Astonishingly, prior computational research has been able to identify putative ADPKD therapeutics, e.g., luteolin from Pedalium murex and the experimental drug lixivaptan.[15].

In order to more thoroughly explore the landscape of possible V2R inhibitors for ADPKD, this research employed a hybrid computational strategy.[16] SwissSimilarity screening, a method which looks for compounds structurally similar to a previously known active compound (in this case, Tolvaptan), was combined with structure-guided blind docking. This hybrid strategy was intended to rank FDA-approved medications both for structural similarity to a previously known V2R antagonist and for beneficial receptor binding interactions.[17] By restricting analysis to approved medications, we hoped to accelerate the search for candidate drugs to repurpose in the treatment of ADPKD, potentially bypassing some of the early-stage pitfalls of new drug development.[18]

METHODOLOGY

This is illustrative of a computer-assisted way of looking for potential drug candidates, most specifically those inhibiting the Vasopressin V2 receptor (V2R) that is implicated in Polycystic Kidney Disease (PKD).[19] They're starting from a known V2R inhibitor, Tolvaptan.

1. Choosing FDA-Approved Drugs: Building a Safe and Working Starting Collection

The initial step was actually one of assembling a given collection of drugs that would stand the best chance of success in future drug development. Rather than fishing around in all manner of hypothetical molecules, the scientists sensibly chose to examine drugs that are already approved by the FDA. FDA-approved drugs have been extensively tested and have a well-documented safety profile in humans, making it less likely to be toxic in later stages. These medications have shown good absorption, distribution, metabolism, and excretion (ADME) characteristics within the human body, which are essential for a drug to work. Reusing known medicines can drastically shorten the timeline for drug discovery and cut the costs involved compared to creating completely new molecules.

2.SwissSimilarity Screening: Identification of Structurally Related Tolvaptan Compounds

In this step, a ligand-based virtual screening technique was employed. The concept here is that molecules that are structurally similar to a known active molecule (Tolvaptan, in this case) will be more likely to have similar biological activity, e.g., inhibiting the V2R. [20] So, there's this nifty little program SwissSimilarity which you can use to compare the Combined CHEBI of the 2D and 3D shapes of all the drugs in the FDA-approved set to the Combined CHEBI of Tolvaptan. Combined CHEBI basically emphasizes the most important features of a molecule that enable it to function biologically, such as how the hydrophobic parts are structured and where the donors and acceptors of the hydrogen bonds are. T he structural similarity of the drugs to Tolvaptan's Combined CHEBI was calculated by the Tanimoto coefficient. The value of the coefficient can range from 0 to 1, with 1 being a perfect similarity. The high functional cut-off of >0.7,

thereby considering only the top 10 compounds with high structural similarity to Tolvaptan. The following analysis was reduced to molecules with a greater likelihood of binding to the V2R in a similar manner. So, Table 1 would have included the top 10 candidate drugs SwissSimilarity came up with and their Tanimoto coefficients, essentially how structurally similar to Tolvaptan they are.[21]

3. Preparing the Protein: Preparing the Molecular Target for Docking.[22]

This was all about making the V2R protein structure well adapted for precise molecular docking simulations. Thus, the three-dimensional structure of the human V2R was accessed from the Protein Data Bank (PDB), essentially a public database of the structural information of large biological molecules. Our PDB code is 4N4H.

Swiss-PDBViewer optimization: Swiss-PDBViewer is computer software for analysing, visualizing, and manipulating protein structures. The researchers utilized it to optimize the structure of V2R. Optimization using such software most likely included:

Adding Missing Atoms: Adding all the atoms found in the protein structure.

Disconnection of Water Molecules and Co-factors (where not applicable): Reducing the system to focus on the protein-ligand interaction. So, in essence, hydrogen atoms don't really show up in X-ray crystal structures, but they're really beneficial for actually being able to know how molecular interactions occur.

Partial Charge Assignment: Suggesting the suitable charges on the protein atoms according to their chemical surroundings.

Active-Site Residue Identification: SCFBio Tools, another computer program, was employed by the researchers to identify important amino acid residues in V2R's binding pocket that might be involved in the binding of a ligand. These residues were Phe105, Lys116, and Gln119, which were identified. This information is important to understand the potential binding interactions of the identified drug candidates.

4. Molecular Docking: Ligand-Protein Interaction Predictions

Molecular docking is a computational approach to the prediction of the binding of a small molecule (ligand, in this instance, the top 10 drug candidates) to a target protein (V2R).[23]

Blind Docking using AutoDock VINA: AutoDock VINA is a popular and successful docking software. The research used "blind docking" in the sense that the whole surface of the V2R protein was scanned as a possible binding site for the ligands. This method prevents bias toward a known binding pocket and enables detection of possible allosteric binding sites (sites other than the main active site but which are nevertheless able to modulate protein function). The docking was conducted using the CB-dock server, a web-server that automates the docking.

Grid Box Covering the Entire Receptor: To do this blind docking thing, we set up a 3D grid box that covers the whole protein structure. This way, the docking algorithm can check out all the different ways the ligands might stick to the protein surface.

Binding Affinities (Vina Scores): Well, AutoDock VINA generates this "Vina score" that essentially indicates how good the ligand binds to the protein. The more negative the Vina score, the stronger and better binding interaction there is typically going on. They recorded these scores for all the top 10 candidate drugs (Table:2)

5. Data Analysis: Ranking and Visualization of Potential Inhibitors

The last step was the filtering and inspection of the docking results to determine the best V2R inhibitors.

Vina Score Ranking: The 10 candidate compounds were ranked based upon their Vina scores. The most negative (lowest) Vina scores were given the highest potential binding affinity for the V2R and were the highest priority for further study.

Visualization of Interactions through Discovery Studio: Discovery Studio is a molecule visualization and analysis tool. The researchers utilized it to analyze the predicted poses of binding of the highest-ranking compounds in the V2R binding site. This helped them to:

Recognize Key Interactions: Imagine the characteristic interactions between the ligand and amino acid residues in the binding site, including hydrogen bonds, hydrophobic interactions, and electrostatic interactions.

Get familiar with Binding Modes: Determine how the ligand accommodates the binding site.

Evaluate the Plausibility of Binding: Determine if the proposed binding mode is energetically favorable and in agreement with any available structure-activity relationships (if available).

In brief, the approach integrates ligand-based virtual screening (structural similarity to a known active molecule) and structure-based virtual screening (molecular docking to predict target protein binding interaction). Beginning with drugs that are approved by the FDA, the study was focused on effectively identifying potential PKD V2R inhibitors with an increased chance of translational success. The application of particular software packages and previously defined parameters in every step provides a systematic and reproducible methodology to in silico drug discovery.

RESULTS

1.Crystal Structure Validation:

The RCSB Protein Data Bank released the crystal structure of human vasopressin V2 receptor (PDB ID:6U1N), and you can view it in figure 1. They ensured that the structure is correct and can be employed for future computational experiments. We carried out our computational screening and docking experiments with the 3D structure of the human vasopressin V2 receptor as our target.

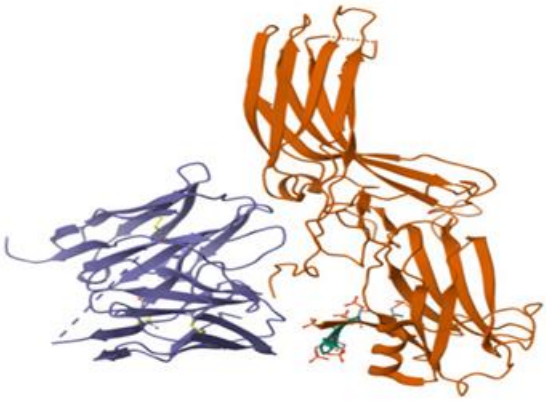


Figure 1: The crystal structure of the human vasopressin V2 receptor (PDB ID: 6U1N)

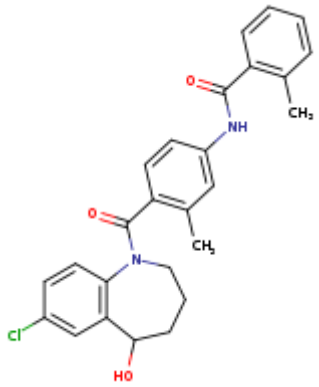
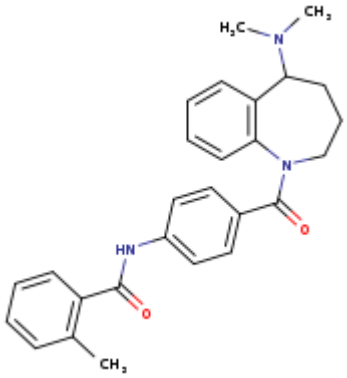
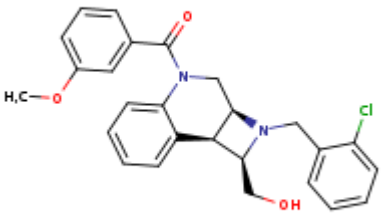
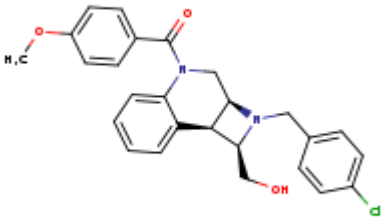
2.Integrated CHEBI and Structure-Based Virtual Screening and Identification of Potential Lead Compounds:

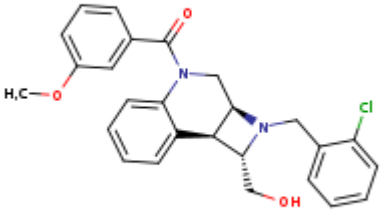
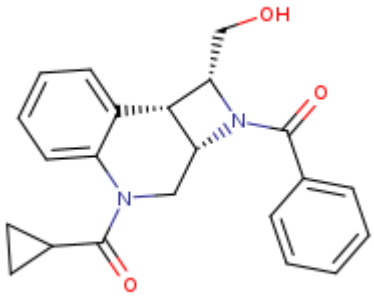
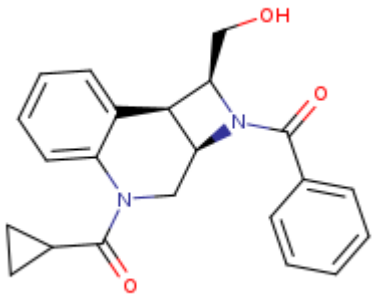
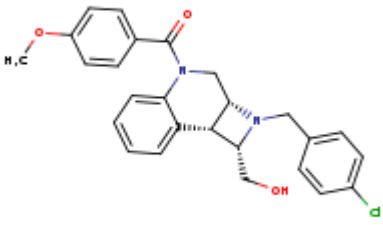
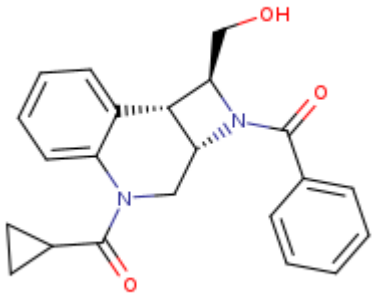
Tolvaptan, a well-documented antagonist of the vasopressin V2 receptor, was employed as a reference molecule to construct a 3-point Combined CHEBI model (as depicted conceptually in Figure 2, adapted for the V2 receptor), constructed based on its essential binding features. The model included crucial aspects like hydrophobic regions, hydrogen bond acceptors, and donors pivotal in binding to the V2 receptor binding site.

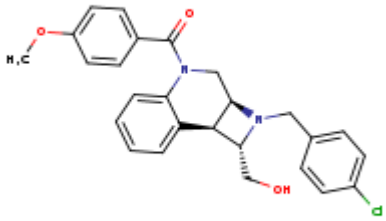
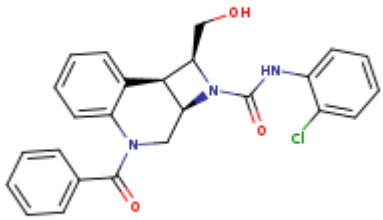
To intensify the stringency and specificity of lead identification, a fused virtual screening strategy was utilized. Initially, a general chemical library, Zinc Drug database, was screened through the Tolvaptan-derived Combined CHEBI model to discover compounds that share complementarity with the V2 receptor

binding site. Then, the highest-ranking compounds in each of the Combined CHEBI screens were docked into the crystal structure of the human vasopressin V2 receptor protein to analyze the binding affinity and interaction modes of the selected compounds in the active site of the receptor.

Table 1: lists a subset of the compounds that were screened using the combined Tolvaptan-derived Combined CHEBI model and molecular docking

Compound ID	Similarity Score	2D Structure
CHEBI:32246 (Tolvaptan)	**1.000**	
CHEBI:31869	0.914	
CHEBI:128672	0.766	
CHEBI:125791	0.757	

CHEBI:127778	0.755	
CHEBI:127456	0.747	
CHEBI:131066	0.752	
CHEBI:130604	**0.745**	
CHEBI:127568	0.737	

CHEBI:129411	0.733	
CHEBI:128957	0.731	

3.Molecular Docking Simulations : Vasopressin V2 Receptor Targeting in Polycystic Kidney Disease-

Molecular docking simulations were conducted to further screen the binding affinities and interactions of the chosen compounds to the 2D & 3D structure of the vasopressin V2 receptor (V2R). Blind docking simulations, as performed using the CB Dock server and AutoDock Vina sophisticated docking software, permitted the ligands to search potential binding sites in the whole V2R structure. The outputs gave insight into the ligand binding modes, most significant interactions, and binding energies in the context of polycystic kidney disease. Ligand binding poses were ranked on the basis of the binding energy calculations derived from the docking simulations. The highest ranking compounds with the best binding affinities and binding interactions with V2R were chosen as lead modulators against V2R in polycystic kidney disease. Structural motifs and binding interactions of these lead compounds were analyzed further to rank the best candidates for experimental confirmation in this disease.

Table 2: Pockets Cavity volume, Center and Cavity size found in V2R

CurPocket ID	Cavity volume (Å3)	Center (x, y, z)	Cavity size (x, y, z)
C5	557	106, 67, 95	25, 25, 25
C1	9069	86, 84, 97	32, 35, 31
C3	958	79, 101, 118	25, 25, 25
C4	658	103, 94, 92	25, 25, 25
C2	4165	95, 82, 82	25, 25, 32

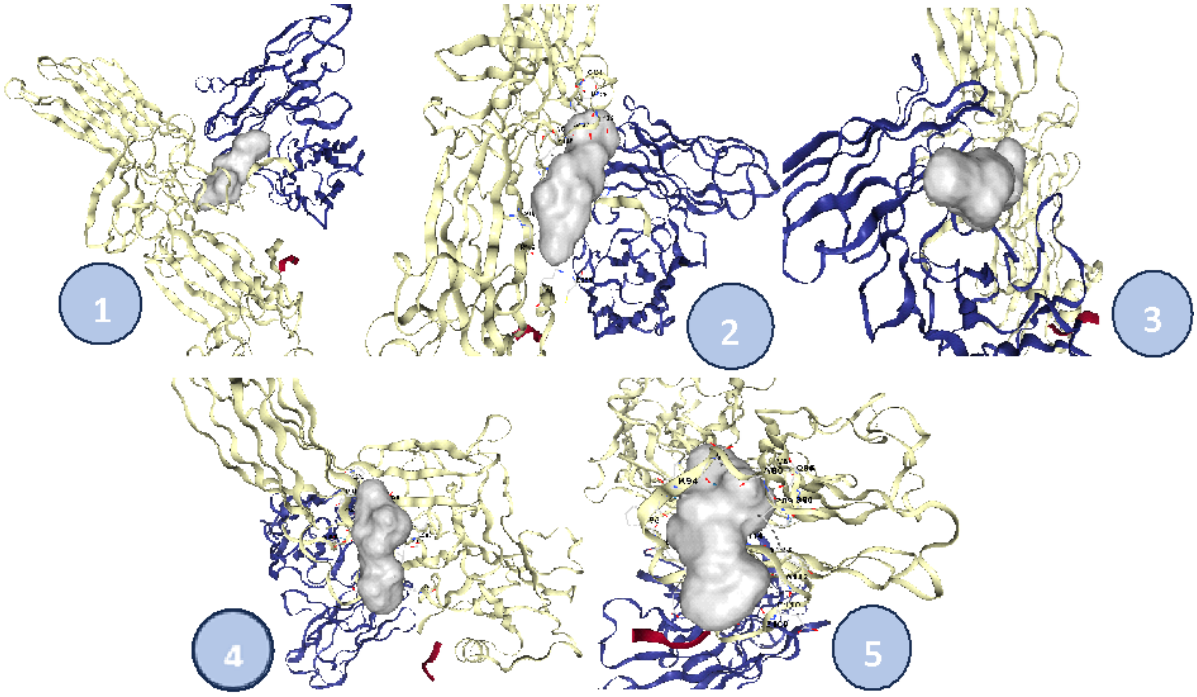


Figure 3: Cavities found in V2 Protein Receptor

Analysis of the data presented here on pockets in V2R is a heterogeneous landscape of structural characteristics. The largest and most significant cavity is C1 (9069 Å³), which must either be equivalent to the orthosteric binding site or a deep transmembrane pocket in V2R. The big docking box hints at being appropriate for larger ligands. The large C2 is also notable (4165 Å³), with a broad, slightly elongated z-axis box, perhaps indicating a narrow side tunnel or side pocket for extended molecules.C3–C5 are smaller, spherical cavities with consistent docking boxes (25 × 25 × 25 Å), suggesting possible allosteric or niche binding pockets.C5, although the smallest (557 Å³), might still provide high specificity for small-molecule modulators, particularly if supported by favorable docking energies.

Table 3: Results of Molecular Docking Studies by CB Dock Server

Sr. No.	**Compound ID**	**Auto Dock Vina Score**
STD	**CHEBI:32246 (Tolvaptan)**	**-9.7**
1	CHEBI:31869	-8.1
2	CHEBI:128672	-8.4
3	CHEBI:125791	-8.4
4	CHEBI:127778	-9.1
5	CHEBI:127456	-7.7
6	CHEBI:131066	-7.6
7	**CHEBI:130604 [(1S,2aR,8bR)-2-[(4-chlorophenyl)methyl]-1-(hydroxymethyl)-1,2a,3,8btetrahydroazeto[2,3-c]quinolin-4-yl]-(4-methoxyphenyl)methanone**	**-10**
8	CHEBI:127568	-8.2
9	CHEBI:129411	-7.4
10	CHEBI:128957	-8.2

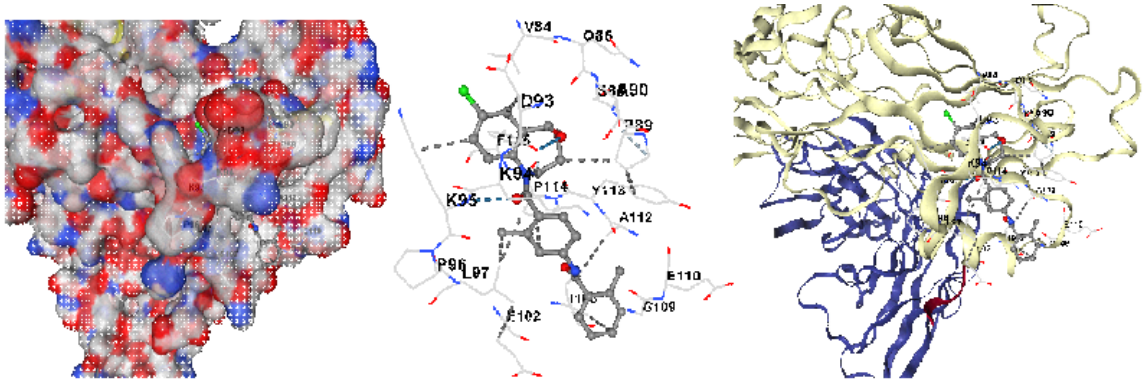


Figure 4: Interactions of V2R and TOLVAPTAN (Auto dock Vina Score -9.7)

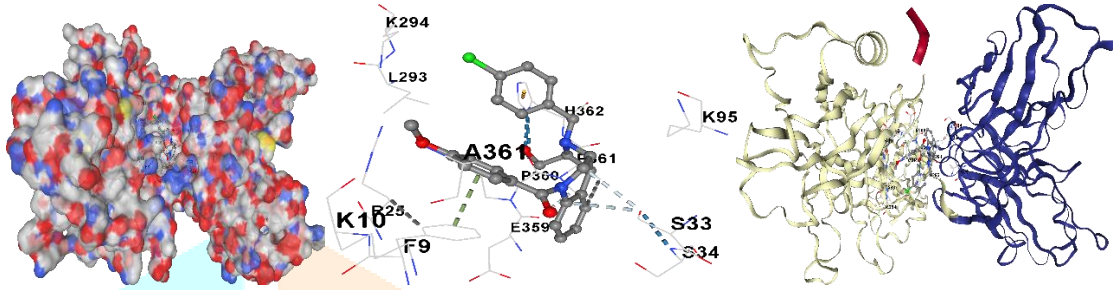


Figure 5: Interactions of V2R and [(1S,2aR,8bR)-2-[(4-chlorophenyl)methyl]-1-(hydroxymethyl)-1,2a,3,8b-tetrahydroazeto[2,3-c]quinolin-4-yl]-(4-methoxyphenyl)methanone (Auto dock Vina Score -10.0 Vs -9.7 of Tolvaptan)

The molecular docking outcome from the CB-Dock Server gives information regarding how many of the different CHEBI compounds interact with possible binding sites in the vasopressin V2 receptor (V2R). The control compound Tolvaptan (CHEBI:32246), which is a recognized V2R antagonist, exhibited a high binding potential with a Vina score of -9.7 kcal/mol, which was used as a reference to compare other candidate molecules. Significantly, ligands CHEBI:130604, CHEBI:127778, CHEBI:128672, and CHEBI:125791 displayed good binding scores of -8.4 to -10.0 kcal/mol, suggesting a consistent affinity for high-affinity interaction across significant binding pockets—presumably within Pocket C1 and Pocket C5, as previously described as having the highest cavity volume and strongest ligand-binding affinity. Specifically, CHEBI:130604 was a leading candidate, beating even the benchmark with a docking score of -10.0 kcal/mol, indicating a possibly greater binding affinity or novel fit in a V2R pocket. The variability of docking scores between these CHEBI compounds indicates varying degrees of binding efficiencies, with a number of compounds having affinities that are similar to or better than the reference molecule, Tolvaptan. These high-affinity molecules are ideal candidates for further study and development as selective V2R modulators. Although individual residue contacts are not described in this dataset, the structural features of Pockets C1 and C5—large cavity volume and favorable geometry—imply that these areas could be hotspots for ligand interaction. Earlier studies suggested conserved residues like ARG104, GLU136, PRO132, and PHE133 in C1, and a denser, potentially allosteric microenvironment in C5.

CONCLUSION

In summary, this study sought to determine possible antagonists of the vasopressin V2 receptor (V2R) by a thorough computational strategy that included molecular docking simulations. The X-ray crystal structure of the V2R was used as the molecular target to explore ligand–receptor interactions, with Tolvaptan (CHEBI:32246), a clinically relevant V2R antagonist, as the reference molecule. A handpicked library of CHEBI compounds were subject to molecular docking using the CB-Dock Server, with docking scores as the only measure to gauge binding affinity. The docking simulations gave ten best-ranking compounds with binding energy values of -7.4 to -10.0 kcal/mol. Of interest was **CHEBI:130604**, which displayed the highest binding affinity with a score of **-10.0 kcal/mol**, surpassing Tolvaptan by a margin, thus making it a potential drug candidate for further development. Other compounds, including CHEBI:127778,

CHEBI:128672, and CHEBI:125791, also showed good docking scores ranging from -8.4 to -9.1 kcal/mol, indicating a similar trend of high-affinity bindings with the V2R structure. Structural analysis of the vasopressin V2 receptor identified five major binding pockets (C1–C5), each with different cavity volumes, docking box sizes, and coordinates. Of these, Pocket C1 had the largest cavity volume and docking size, underlining its importance as a main Ortho steric binding site. Pocket C5, though smaller in size, offered a tight and energetically favorable space that facilitated high-affinity binding, especially by small-molecule ligands. The synergy of ligand-based docking and cavity mapping offered important clues regarding ligand preferences and pocket properties within the V2R. These results offer useful structural and energetic insights to guide the rational design of new V2R inhibitors, especially toward therapeutic uses in treating disorders like hyponatremia and autosomal dominant polycystic kidney disease (ADPKD). Overall, this work sets the stage for experimental validation and further compound optimization of the discovered hit compounds. The structural heterogeneity of the V2R binding pockets highlights the value of a multi-faceted strategy in drug discovery, combining pocket analysis with ligand screening to reveal novel candidates for targeted modulation of vasopressin signalling.

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