Computers in Biology and Medicine 201 (2026) 111428

Contents lists available at ScienceDirect

Computers in Biology and Medicine

journal homepage: www.elsevier.com/locate/compbiomed

Early AI-driven repurposing study of existing drugs towards the vasopressin V2 receptor

Daniela Trisciuzzi [a,1], Ines Angelini [b,1], Nicola Gambacorta [a], Mariangela Centrone [b], Fulvio Ciriaco [c], Lydia Siragusa [d], Annarita Di Mise [e], Marianna Ranieri [b], Giovanna Valenti [b], Nicoletta Guaragnella [b], Cosimo Damiano Altomare [a], Susanna Cotecchia [b], Grazia Tamma [b,2], Orazio Nicolotti [a,2,*]

[a] Department of Pharmacy - Pharmaceutical Sciences, University of Bari Aldo Moro, Italy
[b] Department of Biosciences Biotechnologies and Environment, University of Bari Aldo Moro, Italy
[c] Department of Chemistry, University of Bari Aldo Moro, Italy
[d] Molecular Horizon srl, Via Montelino, 30, 06084, Bettona, PG, Italy
[e] Department of Biotechnology and Biosciences, University of Milan-Bicocca, Milan, Italy

A B S T R A C T

The main goal of this study is the identification of existing drugs that could be repurposed as antagonists of the V2R, a GPCR controlling renal water balance and involved in abnormal cell proliferation, cancer, and renal cyst enlargement. Given its clinical importance, we carried out the reverse screening of a collection of 1882 existing drugs to repurpose them towards V2R by employing PLATO, a home-built target fishing AI-based platform. Five drugs were shortlisted as promising candidates for V2R: cabergoline, clopidogrel, cloxacillin, perphenazine, and zafirlukast. Renal collecting duct MCD4 cells, stably expressing human V2R and AQP2, were used for experimentally testing the effects of the prioritized drugs on V2R responses. FRET studies were conducted to assess whether these drugs affect the DDAVP-induced cAMP responses. Interestingly, zafirlukast, at single-digit nanomolar concentration significantly reduced the DDAVP-dependent cAMP production and water reabsorption, with effects comparable to tolvaptan, a well-known selective V2R antagonist. The molecular rationale behind the observed binding was explained by mapping on the V2R a molecular cleft superimposable to CysLTR1 binding site of zafirlukast. Induced-fit docking simulations demonstrated that zafirlukast engages V2R by adopting a binding conformation closely resembling that of X-ray solved vasopressin. Taken together, our results support the repurposing of zafirlukast as a promising V2R antagonist candidate.

1. Introduction

The vasopressin type 2 receptor (V2R) is a G-protein-coupled receptor (GPCR) playing a paramount action in regulating renal water balance. Upon stimulation, the V2R interacts with the $G_{\alpha s}$ protein by increasing the collecting duct water permeability and by promoting the intracellular trafficking of the water channel aquaporin-2 (AQP2) [1,2]. It is acknowledged that this action is related to cAMP/PKA signaling stimulation. Abnormal vasopressin signals are associated with several diseases, including congestive heart failure, cirrhosis, polycystic kidney disease, syndrome of inappropriate antidiuretic hormone secretion (SIADH), and cancer [3]. Abnormal expression and function of vasopressin receptors have been found in different forms of cancers [4–6]. In renal cell carcinoma, V2R-mediated signaling promoted YAP-dependent cell growth and proliferation [7]. Treatment with the V2R antagonist, mozavaptan, prevented cell proliferation of clear cell renal cell carcinoma (ccRCC) [7]. More recently, the effects of tolvaptan, a selective V2R antagonist, have been investigated in a Murine Xenograft Model of Small Cell Lung Cancer (SCLC), in which the drug showed significant reduction in tumor growth induced by V2R [8]. Abnormal expression and function of V2R have been found in the SIADH, which is a common cause of hyponatremia that leads to electrolyte disorders in elderly and hospitalized patients [9]. The V2R antagonist, tolvaptan, was first approved for the treatment of hyponatremia secondary to SIADH and congestive heart failure (CHF) [10,11]. Lately, tolvaptan has been approved for the treatment of adults with autosomal dominant polycystic kidney disease (ADPKD) [12], although several side effects have been reported, including increased thirst, polyuria, nocturia, polydipsia,

* Corresponding author. Department of Pharmacy - Pharmaceutical Sciences, University of Bari Aldo Moro, Via Orabona 4, 70125, Bari, Italy.
E-mail address: orazio.nicolotti@uniba.it (O. Nicolotti).
[1] These authors contributed equally.
[2] These authors contributed equally.

https://doi.org/10.1016/j.compbiomed.2025.111428
Received 23 October 2025; Received in revised form 5 December 2025; Accepted 23 December 2025
Available online 25 December 2025

and liver toxicity [13]. ADPKD shares many similarities with autosomal recessive polycystic kidney disease (ARPKD), including the intracellular increase in cAMP [14–19]. Nowadays, however, there is no current effective therapy for ARPKD. Therefore, given the adverse effects of known vasopressin receptor antagonists and the importance of the vasopressin/cAMP axis, which has been implicated in several human diseases, an unmet need exists to develop therapeutic strategies targeting this important pathway.

In this challenging scenario, drug repurposing could be an effective option as it would allow us to find new therapeutic uses for existing (i.e., marketed and/or approved) medicines [20,21].

Indeed, drug repurposing offers distinct advantages over conventional *de novo* drug discovery. By leveraging established safety, pharmacokinetic, and manufacturing data, repurposing substantially reduces development time, cost, and attrition risk. This strategy also facilitates a faster clinical application and provides a pragmatic framework for addressing urgent or unmet medical needs.

In this context, the primary objective of the present study is to identify approved drugs that may be repurposed as effective V2R antagonists. By revealing V2R-modulating activity within existing therapeutics, the development of treatments for V2R-related disorders can be expedited, thereby enhancing the likelihood of rapid clinical translation [22]. In this regard, the advent of Artificial Intelligence (AI) and the progress in computing power open unprecedented chances to speed up the drug discovery or repurposing campaigns, enabling the efficient handling of ultra-large chemical libraries made of billions of molecules [23,24].

Nowadays, several examples of AI-driven drug repurposing approaches are available to explore the drug-like chemical space and to unveil latent links bridging known drugs to new therapeutic targets [25–30]. In recent years, a plethora of software and tools have been made available to support drug repurposing campaigns, ranging from open-source packages to commercial suites [31–33]. Among others, PLATO (Polypharmacology pLATform predictiOn) is an easy-to-use technological platform for quantitative polypharmacology profiling [34–36], successfully applied in several recent studies [37–39].

In the present work, a repurposing study was thus carried out by employing the PLATO platform for fishing potential V2R modulators. Starting from a pool of 1882 approved drugs properly retrieved from the ChEMBL database [40], a final set of five drugs were selected for experimental investigation.

Functional studies based on renal Mouse Cortical Collecting Duct (MCD4) cells and Fluorescence Resonance Energy Transfer (FRET) unveiled that zafirlukast, one of these approved drugs, achieved a biological potency comparable to tolvaptan, a well-known selective V2R antagonist. Zafirlukast was able at 1 nM concentration to reduce the 1-deamino-8-D-arginine vasopressin (DDAVP)-dependent cAMP production and water reabsorption.

Computational investigations including molecular docking simulations were then carried out to shed light on the rationale behind the interaction between zafirlukast and V2R. In this context, we also observed a substantial energetic similarity between the crystallographic binding sites of V2R and the Cysteinyl leukotriene receptor 1 (CysLTR1) known to be effectively engaged by zafirlukast, suggesting potential opportunities for drug repurposing [41,42]. Furthermore, molecular docking simulations were performed to elucidate the binding molecular mechanism of zafirlukast towards V2R.

2. Methods

2.1. Cell culture and transfection

Mouse Cortical Collecting Duct cells (MCD4) [43] were grown in Dulbecco's modified Eagle's medium (DMEM/F12) supplemented with 5 % (v/v) fetal bovine serum, 1 % (v/v) L-glutamine, 100 IU/ml penicillin, 100 μg/mL streptomycin, 5 μM dexamethasone. Routinely, cells were tested for Mycoplasma with MycoSPY® Master Mix (DUOTECH SRL, Baranzate, Milano Italy) or by fluorescence staining with DAPI. The cells were maintained at 37 °C in a 5 % CO_2 incubator. MCD4 cells were transfected as previously described [44] using Epac-S-H187, (Plasmid #170348, Addgene). All experiments were performed 24 h after transfection.

2.2. PLATO reverse screening

In silico predictive investigations were carried out by employing a reverse screening based on our home-built PLATO drug discovery platform based on a multi-fingerprint similarity approach for target fishing and bioactivity prediction. PLATO is freely available at https://prometheus.farmacia.uniba.it/plato/ [34–36]. Specifically, the reverse screening workflow was run on a total number of 1882 approved drugs available from the ChEMBL database (release 31) [40]. To expedite and streamline the screening process, all compounds were converted into SMILES (Simplified Molecular Input Line Entry System) strings and screened. The results were stored as a.json file to better manage large volumes of data and information. The reverse VS campaign yielded 185 candidate compounds (available in the Supporting Information), each exhibiting a predicted IC_{50} value toward V2R below 50 nM. Among these, five clinically approved drugs (perphenazine, zafirlukast, cloxacillin, clopidogrel, and cabergoline) were prioritized for subsequent investigations, owing to their acknowledged pharmacological activities in the brain, lung, and kidney, which represent the principal sites of V2R expression.

2.3. Fluorescence Resonance Energy Transfer measurements

To evaluate intracellular changes of cAMP, Fluorescence Resonance Energy Transfer (FRET) experiments were performed as already described [44]. Briefly, cells were transiently transfected with the Epac-S-H187 probe (Epac-S-H187, Addgene plasmid #170348; http://n2t.net/addgene). FRET experiments were performed 24 h after transfection. Cells were perfused with a Ringer solution (140 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, 5 mM Glucose,10 mM HEPES, pH7.4) After recording the basal fluorescence signal, cells were stimulated with DDAVP (100 nM) as a positive and internal control. Alternatively, cells were stimulated with forskolin/IBMX (10^{-5} M/100 μM). In addition, MCD4 cells were treated with cabergoline, clopidogrel, cloxacillin, perphenazine, and zafirlukast at 1 nM, 10 nM, and 100 nM. Alternatively, cells were costimulated with DDAVP and the selected drugs at 1 nM concentration. Detection of FRET was performed on an inverted microscope (Nikon Eclipse TE2000-S) controlled by Metafluor® Software (Molecular Devices, MDS Analytical Technologies, Toronto, Canada).

2.4. Water permeability assay

Osmotic water permeability was measured as previously described [45]. Briefly, MCD4 cells were grown on 25-mm glass coverslips. Cells were left under basal conditions or stimulated with DDAVP (100 nM for 45 min). Alternatively, cells were treated with clopidogrel 1 nM or zafirlukast 1 nM in the absence or the presence of DDAVP (100 nM for 45 min) and loaded with 10 μM membrane-permeable Calcein-AM for 45 min at 37 °C, 5 % CO_2 in DMEM. Measurements were performed using an inverted TE2000-S microscope (Nikon Eclipse microscope, Tokyo, Japan) equipped for single-cell fluorescence measurements and imaging analysis. The Calcein-AM loaded samples were excited at 490 nm. Fluorescence measurements, following isosmotic (140 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, 5 mM Glucose,10 mM HEPES, pH 7.4) or hyperosmotic (isosmotic solution added with 135 mM Mannitol) solutions, were carried out using Metafluor software (Molecular Devices, MDS Analytical Technologies, Toronto, Canada). Time course fluorescence data after perfusing cells with hyper or isosmotic solutions

were recorded every 5 s and over about 3 min. The time constant of cell shrinkage due to the hypertonic stimulus was obtained by fitting data with an exponential function [45].

2.5. Statistical analysis

The one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was applied. Values are shown as means ± Standard Error of the Means (SEMs). The difference of $p < 0.05$ was considered statistically significant.

2.6. Cavities detection and comparison

The 3D dimensional crystallographic structures of V2R (PDB entry 7DW9 [46], resolution equal to 2.60 Å) and CysLTR1 (PDB entry 6RZ5 [42], resolution equal to 2.53 Å) were retrieved from the Protein Data Bank (PDB) [47]. The protein residues were first processed by using the *Fixpdb* tool, and all water molecules and cofactors were filtered. The *Flapsite* algorithm implemented in BioGPS (version 24.01.4) software and licensed by Molecular Discovery Ltd was thus used for the identification of putative cavities in the three-dimensional protein structures [48]. The mapping procedure implied the embedding of the target protein into a 3D grid with a spatial resolution of 1.0 Å and the identification of pocket points through the so-called GRID probe H. The cavities on the V2R and CysLTR1 were thus detected by employing the GRID force field in order to evaluate the type, strength and direction of the molecular interactions established from the cavities. Three GRID probes (that are CRY, N1, and O) were used to quantify hydrophobic, HB acceptor, and HB donor properties. The comparison of the cavities was carried out by looking for the best overlay of the corresponding MIFs. In this respect, the level of similarity was estimated by considering four single probes (i.e., H, CRY, N1, O) and by summing or multiplying their individual contribution to result the values of Global Sum and Global Product, respectively. For details, the interested reader could be referred to Ref. [48].

2.7. Docking protocol calibration

The dimensional cryo-EM structures of V2R (PDB code 7DW9) [46] was firstly refined by using the Protein Preparation Wizard (Schrödinger Suite 2024–1) [49] in order to correct bond order, add hydrogen atoms and possible missing aminoacidic side chains and loops. An energy minimization with Force Fields OPLS-2005 was applied to reduce steric clashes. The 3D conformation of the zafirlukast was processed by using LigPrep (Schrödinger Suite 2024–1) in order to properly generate possible ionization states at a physiological pH value of 7.4. The protonation state of zafirlukast was investigated by using MoKa software (version 4.0.14) [50]. Induced fit docking (IFD) simulation was thus performed by using Schrodinger Suite (Schrödinger Suite 2024–1). A cubic grid with edge of 10 Å centered on cavity residues detected by using BioGPS (version 24.01.4) algorithm (i.e., Thr31, Arg32, Asp33, Leu36, Glu40, Gln92, Gln96, Lys100, Asp103, Arg104, Lys116, Gln119, Met123, Phe178, Asp191, Trp193, Arg202, Val206, Phe287, Gln291, Ala294, Leu302, Glu303, Val308, Met311, Leu312, Leu313 and Ala314) was built [48]. During the initial docking procedure, the van der Waals scaling factor was set to 0.5 for both receptor and zafirlukast. The ligand is docked into the induced-fit receptor structure by using Glide SP and a maximum of 20 poses were considered to be then redocked at SP mode. The Prime refinement step was set by optimizing side chains of residues within 5 Å of the zafirlukast including cavity residues detected by using BioGPS algorithm.

3. Results

3.1. Drugs selection

Based on reverse screening carried out with PLATO, an initial pool of 185 out of 1882 approved drugs properly retrieved from the ChEMBL database (release 31) was selected towards V2R, with predicted $IC_{50} <$ 50 nM (see File_S1.csv of Supporting Information for details). Considering their established pharmacological actions in the brain, lung, and kidney, organs where V2R is predominantly expressed, five candidates were prioritized for experimental evaluation: perphenazine, zafirlukast, cloxacillin, clopidogrel, and cabergoline (Fig. 1).

Cabergoline is a dopamine 2 receptor agonist and was first recommended for use in Parkinson's disease, pituitary cancer, and prolactinomas, and it can also lower blood sugar levels [51]. Clopidogrel is a P2Y12 receptor inhibitor and has antiplatelet effect [52]. Interestingly, clopidogrel has been shown to augment vasopressin responses and increase AQP2 expression [53,54]. Cloxacillin is an anti-staphylococcal antibiotic used to treat serious infections that are resistant to penicillin [55]. Perphenazine is a generic antipsychotic drug well-tolerated in psychiatric patients [56]. Perphenazine is also useful to treat nausea and vomiting due to chemotherapy [57]. Zafirlukast is a leukotriene receptor antagonist, recommended to treat asthma, inflammation, and it also has anti-bacterial actions [58].

3.2. Functional studies to test the effects of possible V2R ligands

To better evaluate the repurposing promise of the five known drugs towards V2R, renal collecting duct MCD4 cells, stably expressing the human V2R, and the AQP2, were used as an experimental cell model [43]. Immunoblotting and confocal analysis confirmed that MCD4 cells express the hV2R (Fig. 2) compared to the mock cell line. FRET studies were performed to test whether the potential V2R modulators identified by reverse VS alter intracellular levels of cAMP. To this end, cells were transiently transfected with the EPAC-based FRET sensors for cAMP, and FRET studies were performed 24 h after transfection. The FRET probe expresses a cAMP-binding sequence of Epac sandwiched between ECFP (donor) and EYFP (acceptor). The binding of cAMP to the cognate interacting sequence results in an intramolecular structural change that increases the distance between the fluorescent donor and the acceptor, thereby decreasing the FRET process [6,59]. In line, Fig. 3 shows that acute stimulation of MCD4 cells with DDAVP, a stable analog of the hormone vasopressin, reduced the FRET signal consistent with a significant increase in intracellular cAMP (DDAVP: 0.71 ± 0.03 vs basal: 1). FRET studies were further used to assess the potential actions, on intracellular cAMP, of the five drugs identified as potential V2R modulators, cabergoline, clopidogrel, cloxacillin, perphenazine, and zafirlukast (Table 1).

The obtained data showed that all these drugs did not affect the basal level of the FRET signal when applied at 1 nM, 10 nM, and 100 nM. Thus, these findings suggest that these potential V2R ligands did not affect the basal level of the intracellular cAMP. Furthermore, co-stimulation of MCD4 cells with DDAVP and cabergoline (1 nM) or cloxacillin (1 nM), or perphenazine (1 nM) reduced the FRET signal similarly to DDAVP alone, suggesting that these drugs did not alter the intracellular cAMP response induced by DDAVP. Additionally, compared to DDAVP stimulation, the FRET signal was significantly reduced when cells were co-stimulated with DDAVP and clopidogrel, likely suggesting that clopidogrel further increased the intracellular level of cAMP. In this respect, osmotic water permeability measurements (Fig. 4) revealed that compared to cells left under control conditions, treatment with clopidogrel significantly increased the intracellular water transport, similarly to cells stimulated with DDAVP (clopidogrel: 2.44 ± 0.13; n = 127 cells; DDAVP: 2.56 ± 0.12; n = 250 cells vs control: 1.00 ± 0.03; n = 355 cells).

To test whether the clopidogrel-induced water transport was

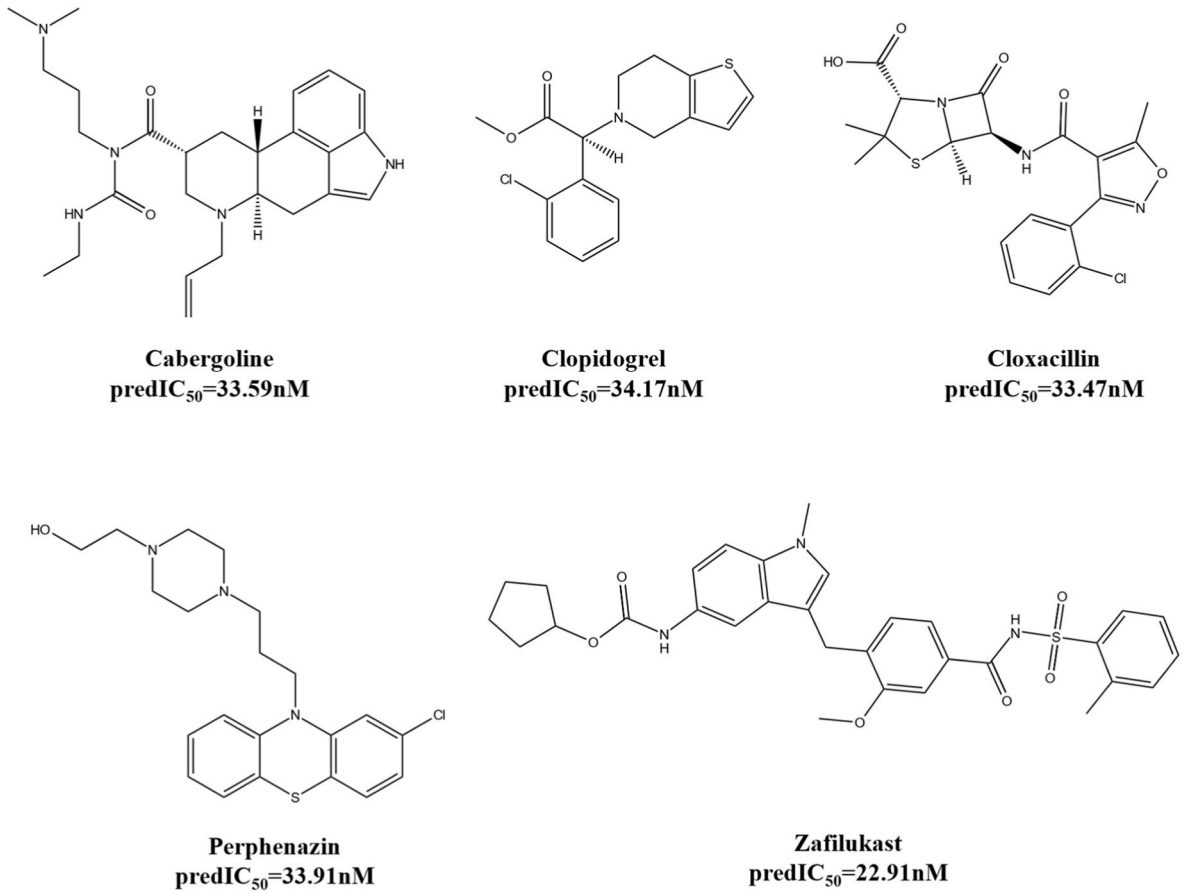

Fig. 1. Prioritized five drugs towards V2R based on reverse VS campaign. The IC_{50} values predicted by PLATO are also reported.

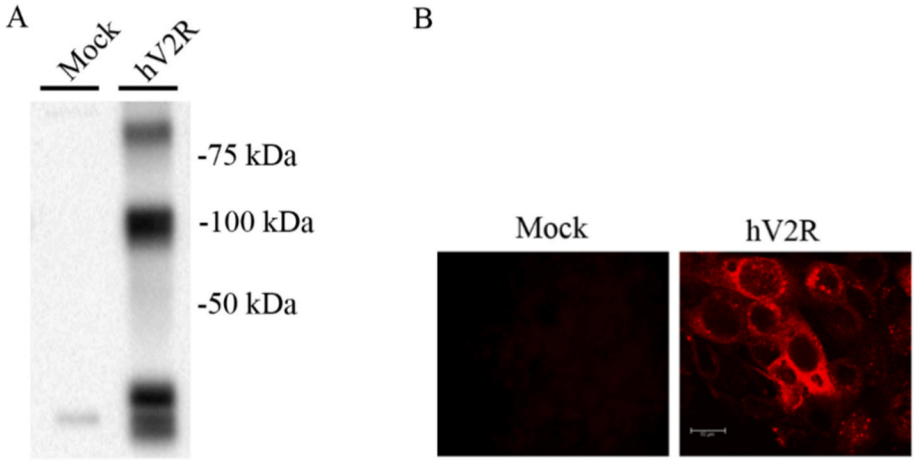

Fig. 2. *V2R expression in MCD4 cells.* (A) Immunoblotting experiments showed that MCD4 cells express the hV2R compared to mock cells. (B) Immunofluorescence analysis confirmed the expression of hV2R in MCD4 cells.

mediated by V2R signaling, cells were stimulated with clopidogrel in the presence of tolvaptan (10 nM for 45 min). Data showed that treatment with tolvaptan did not affect the intracellular water reabsorption induced by clopidogrel (clopidogrel/tolvaptan: 2.66 ± 0.15; n = 102 cells vs clopidogrel: 2.44 ± 0.13; n = 127 cells), suggesting that the effect of clopidogrel on intracellular water transport is independent of V2R activation and cAMP generation. Indeed, co-stimulation with DDAVP and clopidogrel increased the intracellular water transport compared to the treatment with DDAVP alone (clopidogrel/DDAVP: 3.14 ± 0.21; n = 103 cells vs DDAVP: 2.56 ± 0.13; n = 250 cells). In contrast, zafirlukast prevented DDAVP-induced FRET reduction, similar to the response obtained when MCD4 cells were exposed to tolvaptan (zafirlukast 1 nM + DDAVP: 0.99 ± 0.01; tolvaptan + DDAVP: 1.02 ± 0.02). Specifically, Fig. 5A revealed that zafirlukast did not alter the basal level of cAMP when applied at 1 nM, 10 nM, and 100 nM (Table 1). In addition, no reduction in FRET signal was observed when cells were co-stimulated with zafirlukast and DDAVP. Conversely, a significant decrease in the FRET signal, consistent with increased cAMP, was observed following treatment with forskolin and iso-butylmethylxanthine (FK/IBMX, 10^{-5} M/100 μM), which increases

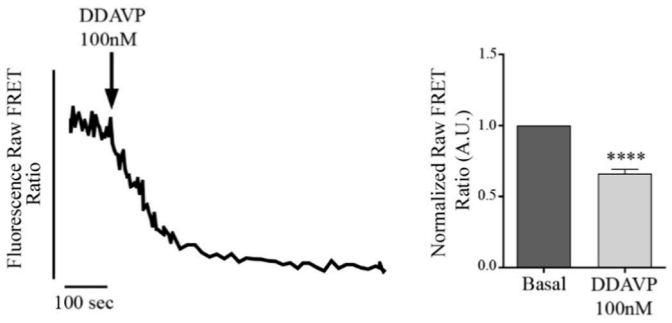

Fig. 3. *Evaluation of intracellular changes in cAMP elicited by DDAVP.* A representative time course of the fluorescence raw FRET ratio. Stimulation with DDAVP resulted in a significant decrease in the FRET ratio consistent with an increase in intracellular cAMP. Data are shown as means ± SEMs (****$p <$ 0.0001 vs basal; DDAVP: 0.71 ± 0.03 vs basal: 1).

Table 1
FRET studies were applied to the five selected potential V2R modulators. Data are shown as means ± SEMs[†].

		1 nM	10 nM	100 nM	1 nM + 100 nM DDAVP
Cabergoline		0.90 ± 0.02**n = 37	0.87 ± 0.03***n = 30	0.88 ± 0.04**n = 30	0.54 ± 0.03****n = 16
Clopidogrel		0.98 ± 0.01 n = 24	1.00 ± 0.01 n = 25	1.02 ± 0.02 n = 15	0.42 ± 0.03****n = 9
Cloxacillin		0.99 ± 0.03 n = 8	0.93 ± 0.02 n = 11	0.83 ± 0.03**n = 11	0.56 ± 0.04****n = 7
Perphenazine		1.01 ± 0.01 n = 16	1.01 ± 0.02 n = 16	1.02 ± 0.03 n = 16	0.65 ± 0.05****n = 17
Zafirlukast		1.03 ± 0.02 n = 42	1.06 ± 0.04 n = 6	1.17 ± 0.09 n = 6	0.99 ± 0.01 n = 32
Basal	1.00 n = 169				
DDAVP	0.71 ± 0.03****n = 64				
FK + IBMX	0.67 ± 0.03****n = 64				

[†]*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ *vs* basal.

intracellular cAMP by inducing adenylate cyclase and inhibiting phosphodiesterases, respectively. Furthermore, the FRET ratio was also evaluated when cells were first stimulated with DDAVP alone (Fig. 5B). Importantly, DDAVP failed to increase cAMP only when zafirlukast was added. In contrast, FK/IBMX significantly increased cAMP by acting as a downstream effector of the V2R pathway. Data and statistics are shown in the bar plot (Fig. 5C).

Functional studies were carried out to assess whether the zafirlukast-dependent impairment of DDAVP-induced cAMP generation reduced the AQP2-regulated water reabsorption (Fig. 6). Importantly, treatment with zafirlukast abolished the stimulatory effect of DDAVP-regulated water permeability, similarly to the action elicited by treatment with tolvaptan, likely suggesting that zafirlukast is acting as a negative modulator of the V2R-regulated signaling.

3.3. *In silico drug repurposing of zafirlukast toward V2R*

The identification of target binding sites as well as their structural comparison are increasingly important in drug design. In this respect, BioGPS is a software based on a semiautomated approach able to characterize and align them directly by comparing GRID-MIFs [48].

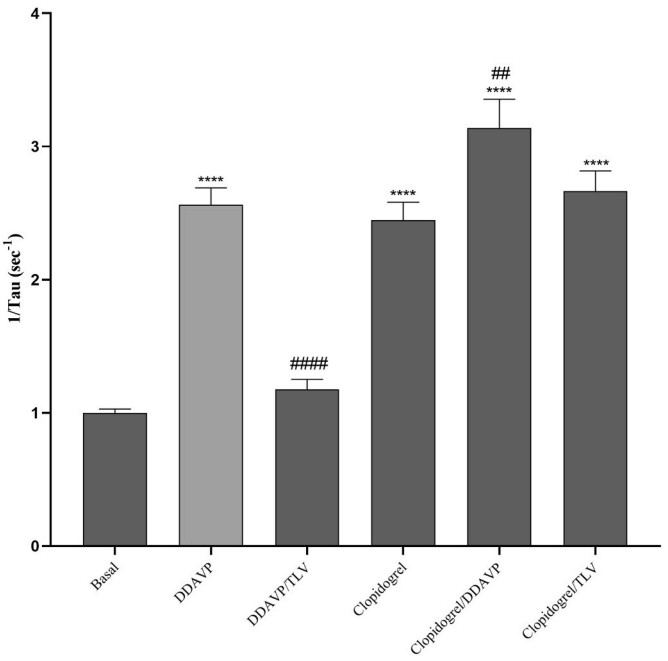

Fig. 4. *Water permeability measurement under clopidogrel treatment.* Cells were grown and treated as described in the Methods section. The time course of fluorescence changes in calcein-loaded cells indicates that clopidogrel significantly increased the intracellular water transport, similarly to cells stimulated with DDAVP. Co-treatment with DDAVP/clopidogrel significantly increased the osmotic water permeability compared to cells stimulated with only DDAVP. Data are shown as means ± SEMs (****$p < 0.0001$ vs basal; ####$p < 0.0001$ *vs* DDAVP).

Zafirlukast is a highly selective antagonist of CysLT1R, a pharmacological target involved in the treatment of asthma and inflammation [58]. In this context, the crystallographic binding site of CysLTR1 co-crystallized with zafirlukast available in Protein Data Bank (PDB entry 6RZ5 [42]), was superimposed with crystallographic binding site of V2R by looking for the best overlay of the corresponding MIFs, in order to investigate the potential activity of zafirlukast towards the V2R. The superimposition of MIFs for the two cavities is depicted in Fig. 7, whereas the GRID probe similarity scores are summarized in Table S1 of the Supporting Information. For the sake of completeness, further structural details about the residues of the two mapped cavities are reported Fig. S1 of the Supporting Information.

Despite the different folding of the two proteins, the cavities shared a similar shape with an overlap of about 79 %. Looking at the similarity scores, the largest values correspond to polar interactions established by HBD (GRID probe N1) and HBA (GRID probe O) probes. Overall, the extent of structural similarity (Global-Sum = 0.680) between the two crystallographic active sites would suggest that zafirlukast could be repurposed as a potential modulator of V2R. Noteworthy, IFD simulation also revealed that zafirlukast would be a good binder towards V2R, as a good overlap was observed between its predicted docking pose and the co-crystallized vasopressin, as depicted in Fig. 8. For the sake of clarity, all cavity residues detected by BioGPS were considered flexible in order to explore in detail the molecular recognition with zafirlukast. As far as the molecular interactions are concerned, the obtained top-docking pose of zafirlukast (docking score equal to −7.695 kcal/mol) showed two π-π contacts between the indole group and Phe178 and Trp193 of V2R at a distance of 5.3 Å and 4.6 Å, respectively. The zafirlukast was also able to engage HBs with the backbone Lys116 and Gln96 of V2R at a distance of 2.3 Å and 1.9 Å, respectively. In addition, the nitrogen of sulfonamide group also established salt bridge with Lys100 of V2R. A comprehensive 2D interaction diagram of zafirlukast

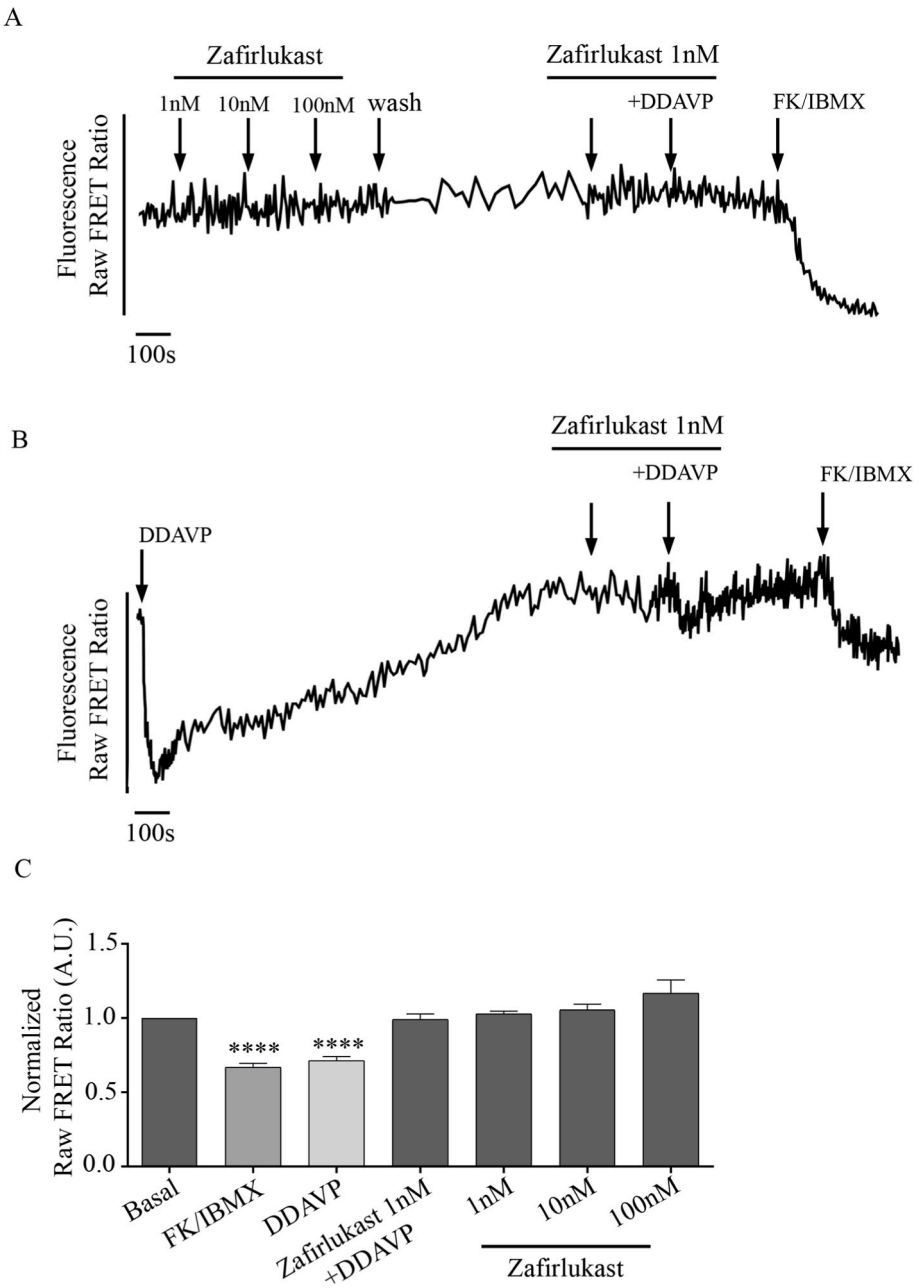

Fig. 5. *Evaluation of intracellular changes in cAMP elicited by zafirlukast.* (A) A representative time course of the fluorescence raw FRET ratio. Incubation with zafirlukast at 1 nM,10 nM and 100 nM did not alter the FRET ratio. Moreover, DDAVP failed in reducing the FRET ratio when cotreated with zafirlukast. FK/IBMX significantly decreased the FRET ratio. (B) A representative time course of the fluorescence raw FRET ratio showing the effect of DDAVP, zafirlukast + DDAVP and FK/IBMX. (C) A bar plot showing the normalized raw FRET data. Data are shown as means ± SEMs (****$p < 0.0001$ *vs* basal).

with V2R is reported in Fig. S2 of the Supporting Information.

4. Discussion

To date, the non-peptide V2R antagonist tolvaptan remains the only approved therapy for the treatment of ADPKD and is also used in several cancer-related cases of SIADH. Tolvaptan functions by binding to the V2R, inhibiting the activation of the $G_{\alpha s}$-dependent pathway, thereby preventing the intracellular generation of cAMP. However, its clinical use is frequently limited by adverse effects such as excessive thirst and polyuria, and long-term treatment carries the risk of severe and potentially fatal liver toxicity. These limitations highlight the urgent need for safer therapeutic alternatives. Consequently, this study aims to identify already-approved drugs that can be repurposed as V2R antagonists, offering a faster and potentially safer route to novel treatments.

In silico drug repurposing approaches enable efficient exploration of the drug-like chemical space, uncovering latent connections between existing drugs and new therapeutic targets. In this study, we employed PLATO, a free and easy-to-use technological platform to identify potential modulators for repurposing analysis towards V2R. Basically, by using a reverse VS strategy, PLATO was able to select 185 out of 1882 approved drugs as promising candidates with a precited $IC_{50} < 50$ nM towards V2R. Five known drugs (i.e., perphenazine, zafirlukast, cloxacillin, clopidogrel and cabergoline) were finally shortlisted for experimental testing being already acknowledged their role in organs like brain, lung and kidney normally expressing V2R.

In particular, the selected five known drugs were functionally tested in renal collecting duct MCD4 cells stably expressing human V2R.

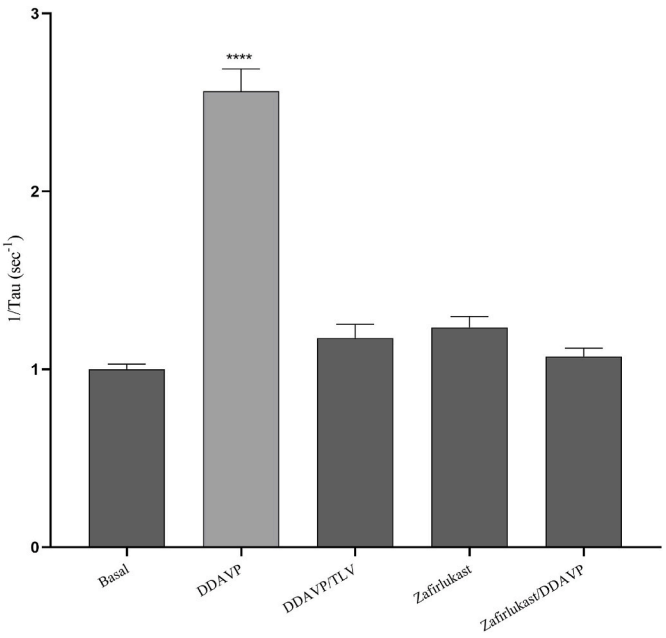

Fig. 6. *Water permeability measurement under zafirlukast treatment.* Cells were grown and treated as described in the Methods section. The time course of fluorescence changes in calcein-loaded cells indicates that in the presence of zafirlukast, DDAVP failed to promote cell permeability. Data are shown as means ± SEMs (****$p < 0.0001$ *vs* basal).

Specifically, FRET studies revealed that treatment with perphenazine or cloxacillin or cabergoline did not affect the intracellular cAMP generation in the absence or presence of DDAVP, likely suggesting that these drugs did not bind or functionally interact with V2R. Conversely, clopidogrel treatment potentiated the DDAVP response in terms of cAMP production, despite no changes in intracellular cAMP being evoked by clopidogrel treatment alone. Clopidogrel is a selective inhibitor of the P2Y12 receptor that plays key roles in platelet aggregation, inflammation, and renal water reabsorption. Particularly, in the renal collecting duct, clopidogrel mitigates the lithium-induced water diuresis by promoting urine concentration and AQP2 expression and function. Functional studies showed that incubation with clopidogrel alone significantly increased the osmotic water reabsorption in MCD4 cells. Notably, this increase was not prevented by tolvaptan exposure, suggesting that clopidogrel may increase the osmotic water reabsorption independently of the V2R signal transduction pathway. Accordingly, cotreatment with clopidogrel and DDAVP significantly increased the osmotic permeability compared to cells treated with DDAVP alone. These findings revealed that clopidogrel can modulate AQP2-dependent water reabsorption independently of the V2R binding and signaling. Conversely, treatment with zafirlukast prevented the DDAVP-induced cAMP generation and consequently the DDAVP-regulated water permeability, similarly to the action evoked by tolvaptan. Zafirlukast is a leukotriene receptor antagonist approved for the treatment of asthma in adults and children [60]. Leukotrienes are derived from arachidonic acid metabolism and are important inflammatory mediators. Following oral administration, zafirlukast is rapidly absorbed. This drug mainly binds to plasma proteins and has low permeability through the blood-brain barrier. Zafirlukast is mostly metabolized in the liver [61], and only 10 % is excreted in the urine [62], likely suggesting that only a small portion may reach renal tubules. However, in the kidney, leukotrienes decrease renal blood flow and glomerular filtration rate (GFR) [63]. Recent findings reported that zafirlukast has been repurposed for renoprotection action in ischemia-reperfusion injury [64], in which vasopressin plays a modulatory effect [65]. In this report, we find that zafirlukast prevented the DDAVP-induced cAMP generation and water reabsorption, possibly by functionally interacting with V2R signaling. Importantly, zafirlukast is a selective antagonist inhibiting the effect of CysLT1R that is coupled to Gi [66]. At the molecular level, inhibition of Gi, would lead to a relevant increase of intracellular cAMP that was not found in our study. In addition, CysLT1R is not expressed in the renal collecting duct cells [67]. Our findings, therefore, strongly suggest that in MCD4 cells, zafirlukast is not acting on its classical cognate CysLT1R, even if it cannot be excluded that zafirlukast is modulating other intracellular signaling crosstalking with the DDAVP-dependent

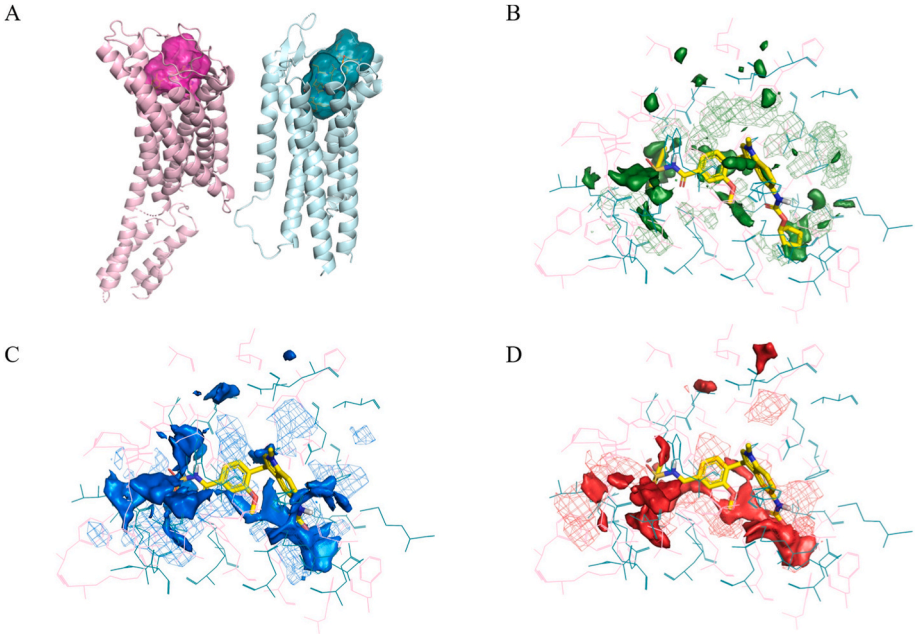

Fig. 7. V2R (PDB entry 7DW9) [46] and CysLTR1 (PDB entry 6RZ5) [42] cavities predicted by BioGPS are depicted in cyan and pink surface and are shown in Panel A. Superimposition of GRID-MIFs for the two cavities representing hydrophobic, HB donor and HB acceptor interactions colored in green, blue and red are shown in Panel B, C and D, respectively. The energetic isocontours are displayed as mesh/surface, generated from the V2R/CysLTR1 crystal structures. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

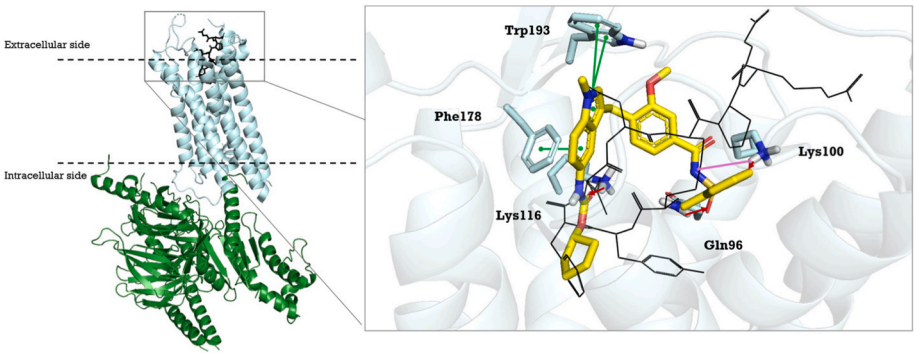

Fig. 8. IFD pose of Zafirlukast (yellow sticks) towards V2R (cyan cartoon) in complex with an G_s protein (dark green cartoon) (PDB = 7DW9) [46]. Green, red and pink arrows indicate π− π stacking, HBs and salt bridge, respectively. Vasopressin is depicted as black lines. For the sake of completeness, all molecular interactions observed in IFD simulation were automatically flagged by GLIDE software. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

pathway. Notably, the *in silico* investigation unveiled a significant similarity between the crystallographic cavities of the V2R and CysLT1R, demonstrating approximately 79 % of shape similarity despite the different overall folding of the two proteins. Overall, the structural and energetic similarity expressed in terms of GRID-MIFs with a global-sum parameter equal to 0.680 prompted us to hypothesize that zafirlukast could be effectively repurposed towards V2R. Furthermore, our structure-based studies demonstrated that a good overlap exists between the docking and crystallographic poses of zafirlukast and vasopressin at the interface of V2R binding site.

Indeed, *ex vivo* and *in vivo* studies would strengthen our results, thus confirming zafirlukast as a V2R antagonist. In particular, future studies are needed to test renal targeting in more detail, given the very low renal excretion. Finally, evaluating zafirlukast against current V2R antagonists in preclinical models of SIADH and ADPKD could clarify its potential use in other still incurable disorders, including ARPKD.

CRediT authorship contribution statement

Daniela Trisciuzzi: Visualization, Validation, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Ines Angelini:** Visualization, Validation, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Nicola Gambacorta:** Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Mariangela Centrone:** Methodology, Investigation, Data curation, Conceptualization. **Fulvio Ciriaco:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lydia Siragusa:** Software, Methodology, Investigation, Data curation, Conceptualization. **Annarita Di Mise:** Investigation, Formal analysis, Data curation. **Marianna Ranieri:** Investigation, Formal analysis, Data curation. **Giovanna Valenti:** Investigation, Formal analysis, Data curation. **Nicoletta Guaragnella:** Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Cosimo Damiano Altomare:** Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Susanna Cotecchia:** Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Grazia Tamma:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Orazio Nicolotti:** Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

Ethics statement

This research was conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Funding

N.Ga., F.C., N.Gu., C.D.A., S.C., G.T. and O.N. gratefully thank Horizon Europe Seeds “L’intelligenza artificiale a tutela della salute in età pediatrica. Implementazione di una piattaforma digitale per il design di farmaci pediatrici sicuri”, Università degli Studi di Bari (Bari, Italy) (CUP: H99J21017390006).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compbiomed.2025.111428.

References

[1] C. Daly, A.A. Guseinov, H. Hahn, A. Wright, I.G. Tikhonova, A.R.B. Thomsen, B. Plouffe, β-Arrestin-Dependent and -Independent endosomal G protein activation by the vasopressin type 2 receptor, eLife 12 (2023), https://doi.org/10.7554/eLife.87754. RP87754.
[2] M. Centrone, M. Ranieri, A. Di Mise, M. D'Agostino, M. Venneri, A. Ferrulli, G. Valenti, G. Tamma, AQP2 trafficking in health and diseases: an updated overview, Int. J. Biochem. Cell Biol. 149 (2022) 106261, https://doi.org/10.1016/j.biocel.2022.106261.
[3] G. Valenti, G. Tamma, The Vasopressin-Aquaporin-2 pathway syndromes, Handb. Clin. Neurol. 181 (2021) 249–259, https://doi.org/10.1016/B978-0-12-820683-6.00018-X.
[4] P.L. Dahia, A. Ahmed-Shuaib, R.A. Jacobs, S.L. Chew, J. Honegger, R. Fahlbusch, G.M. Besser, A.B. Grossman, Vasopressin receptor expression and mutation analysis in corticotropin-secreting tumors, J. Clin. Endocrinol. Metab. 81 (5) (1996) 1768–1771, https://doi.org/10.1210/jcem.81.5.8626831.
[5] W.G. North, M.J. Fay, J. Du, MCF-7 breast cancer cells express normal forms of all vasopressin receptors plus an abnormal V2R, Peptides 20 (7) (1999) 837–842, https://doi.org/10.1016/s0196-9781(99)00070-4.
[6] M. Centrone, M. D'Agostino, M. Ranieri, M.G. Mola, P. Faviana, P.V. Lippolis, D. A. Silvestris, M. Venneri, A. Di Mise, G. Valenti, G. Tamma, dDAVP downregulates the AQP3-Mediated glycerol transport via V1aR in human Colon HCT8 cells, Front. Cell Dev. Biol. 10 (2022) 919438, https://doi.org/10.3389/fcell.2022.919438.
[7] A. Jamadar, N. Dwivedi, S. Mathew, J.P. Calvet, S.M. Thomas, R. Rao, Vasopressin receptor Type-2 mediated signaling in renal cell carcinoma stimulates stromal fibroblast activation, Int. J. Mol. Sci. 23 (14) (2022) 7601, https://doi.org/10.3390/ijms23147601.
[8] L. Naldi, B. Fibbi, S. Polvani, C. Cirillo, F. Pasella, F. Bartolini, F. Romano, A. Fanelli, A. Peri, G. Marroncini, The vasopressin receptor antagonist tolvaptan counteracts tumor growth in a murine xenograft model of small cell lung cancer, Int. J. Mol. Sci. 25 (15) (2024) 8402, https://doi.org/10.3390/ijms25158402.
[9] W. Fenske, B. Allolio, The syndrome of inappropriate secretion of antidiuretic hormone: diagnostic and therapeutic advances, Horm. Metab. Res. 42 (10) (2010) 691–702, https://doi.org/10.1055/s-0030-1255117.
[10] M. Mele, A. Mele, P. Imbrici, F. Samarelli, R. Purgatorio, G. Dinoi, M. Correale, O. Nicolotti, A. De Luca, N.D. Brunetti, A. Liantonio, N. Amoroso, Pleiotropic

effects of direct oral anticoagulants in chronic heart failure and atrial fibrillation: machine learning analysis, Molecules 29 (11) (2024) 2651, https://doi.org/10.3390/molecules29112651.
[11] M. Mele, P. Imbrici, A. Mele, M.V. Togo, G. Dinoi, M. Correale, N.D. Brunetti, O. Nicolotti, A. De Luca, C.D. Altomare, A. Liantonio, N. Amoroso, Short-term anti-remodeling effects of gliflozins in diabetic patients with heart failure and reduced ejection fraction: an explainable artificial intelligence approach, Front. Pharmacol. 14 (2023), https://doi.org/10.3389/fphar.2023.1175606.
[12] L. Sans-Atxer, D. Joly, Tolvaptan in the treatment of autosomal dominant polycystic kidney disease: patient selection and special considerations, Int. J. Nephrol. Renovascular Dis. 11 (2018) 41–51, https://doi.org/10.2147/IJNRD.S125942.
[13] V.E. Torres, A.B. Chapman, O. Devuyst, R.T. Gansevoort, J.J. Grantham, E. Higashihara, R.D. Perrone, H.B. Krasa, J. Ouyang, F.S. Czerwiec, TEMPO 3:4 Trial Investigators, Tolvaptan in patients with autosomal dominant polycystic kidney disease, N. Engl. J. Med. 367 (25) (2012) 2407–2418, https://doi.org/10.1056/NEJMoa1205511.
[14] M.I. Parker, A.S. Nikonova, D. Sun, E.A. Golemis, Proliferative signaling by ERBB proteins and RAF/MEK/ERK effectors in polycystic kidney disease, Cell. Signal. 67 (2020) 109497, https://doi.org/10.1016/j.cellsig.2019.109497.
[15] S.A. Orellana, W.E. Sweeney, C.D. Neff, E.D. Avner, Epidermal growth factor receptor expression is abnormal in murine polycystic kidney, Kidney Int. 47 (2) (1995) 490–499, https://doi.org/10.1038/ki.1995.62.
[16] W.E. Sweeney, R.O. von Vigier, P. Frost, E.D. Avner, Src inhibition ameliorates polycystic kidney disease, J. Am. Soc. Nephrol. 19 (7) (2008) 1331–1341, https://doi.org/10.1681/ASN.2007060665.
[17] M. Kugita, K. Nishii, T. Yamaguchi, A. Suzuki, Y. Yuzawa, S. Horie, E. Higashihara, S. Nagao, Beneficial effect of combined treatment with octreotide and pasireotide in PCK rats, an orthologous model of human autosomal recessive polycystic kidney disease, PLoS One 12 (5) (2017) e0177934, https://doi.org/10.1371/journal.pone.0177934.
[18] B. Padhy, J. Xie, R. Wang, F. Lin, C.-L. Huang, Channel function of Polycystin-2 in the endoplasmic reticulum protects against autosomal dominant polycystic kidney disease, J. Am. Soc. Nephrol. 33 (8) (2022) 1501–1516, https://doi.org/10.1681/ASN.2022010053.
[19] M.L. Rosenberg, A. Yaneff, G.M. Ferradás, M.P. Villafañe Tapia, C.A. Davio, N. P. Goette, S.G. Vlachovsky, R.N. Peroni, E.M. Oddo, P.J. Azurmendi, Total and extracellular vesicle cAMP contents in urine are associated with Autosomal Dominant Polycystic Kidney Disease (ADPKD) progression, Life (Basel) 13 (9) (2023) 1817, https://doi.org/10.3390/life13091817.
[20] L. Pinzi, N. Bisi, G. Rastelli, How drug repurposing can advance drug discovery: challenges and opportunities, Front. Drug Discov. (2024) 4, https://doi.org/10.3389/fddsv.2024.1460100.
[21] S. Pushpakom, F. Iorio, P.A. Eyers, K.J. Escott, S. Hopper, A. Wells, A. Doig, T. Guilliams, J. Latimer, C. McNamee, A. Norris, P. Sanseau, D. Cavalla, M. Pirmohamed, Drug repurposing: progress, challenges and recommendations, Nat. Rev. Drug Discov. 18 (1) (2019) 41–58, https://doi.org/10.1038/nrd.2018.168.
[22] P. Zhan, B. Yu, L. Ouyang, Drug repurposing: an effective strategy to accelerate contemporary drug discovery, Drug Discov. Today 27 (7) (2022) 1785–1788, https://doi.org/10.1016/j.drudis.2022.05.026.
[23] K. Zhang, X. Yang, Y. Wang, Y. Yu, N. Huang, G. Li, X. Li, J.C. Wu, S. Yang, Artificial intelligence in drug development, Nat. Med. 31 (1) (2025) 45–59, https://doi.org/10.1038/s41591-024-03434-4.
[24] J. Lyu, J.J. Irwin, B.K. Shoichet, Modeling the expansion of virtual screening libraries, Nat. Chem. Biol. 19 (6) (2023) 712–718, https://doi.org/10.1038/s41589-022-01234-w.
[25] L. Cortial, V. Montero, S. Tourlet, J. Del Bano, O. Blin, Artificial intelligence in drug repurposing for rare diseases: a mini-review, Front Med (Lausanne) 11 (2024) 1404338, https://doi.org/10.3389/fmed.2024.1404338.
[26] A. Singh, Artificial intelligence for drug repurposing against infectious diseases, Artificial Intelligence Chem. 2 (2) (2024) 100071, https://doi.org/10.1016/j.aichem.2024.100071.
[27] Z. Tanoli, M. Vähä-Koskela, T. Aittokallio, Artificial intelligence, machine learning, and drug repurposing in cancer, Expet Opin. Drug Discov. 16 (9) (2021) 977–989, https://doi.org/10.1080/17460441.2021.1883585.
[28] J.-H. Gan, J.-X. Liu, Y. Liu, S.-W. Chen, W.-T. Dai, Z.-X. Xiao, Y. Cao, DrugRep: an automatic virtual screening server for drug repurposing, Acta Pharmacol. Sin. 44 (4) (2023) 888–896, https://doi.org/10.1038/s41401-022-00996-2.
[29] K. Abbasi, P. Razzaghi, A. Gharizadeh, A. Ghareyazi, A. Dehnad, H.R. Rabiee, M.R. K. Mofrad, Computational drug design in the AI era: a systematic review of molecular representations, generative architectures, and performance assessment, Pharmacol. Rev. (2025) 100095, https://doi.org/10.1016/j.pharmr.2025.100095.
[30] R. Norouzi, R. Norouzi, K. Abbasi, R. Norouzi, P. Razzaghi, DFT_ANPD: a dual-feature two-sided attention network for anticancer natural products detection, Comput. Biol. Med. 194 (2025) 110442, https://doi.org/10.1016/j.compbiomed.2025.110442.
[31] Z. Wan, X. Sun, Y. Li, T. Chu, X. Hao, Y. Cao, P. Zhang, Applications of artificial intelligence in drug repurposing, Adv. Sci. (Weinh.) 12 (14) (2025) e2411325, https://doi.org/10.1002/advs.202411325.
[32] V.O.K. Li, Y. Han, T. Kaistha, Q. Zhang, J. Downey, I. Gozes, J.C.K. Lam, DeepDrug as an expert guided and AI driven drug repurposing methodology for selecting the lead combination of drugs for alzheimer's disease, Sci. Rep. 15 (1) (2025) 2093, https://doi.org/10.1038/s41598-025-85947-7.
[33] T. Mohammadzadeh-Vardin, A. Ghareyazi, A. Gharizadeh, K. Abbasi, H.R. Rabiee, DeepDRA: drug repurposing using multi-omics data integration with autoencoders, PLoS One 19 (7) (2024) e0307649, https://doi.org/10.1371/journal.pone.0307649.
[34] F. Ciriaco, N. Gambacorta, D. Trisciuzzi, O. Nicolotti, PLATO: a predictive drug discovery web platform for efficient target fishing and bioactivity profiling of small molecules, Int. J. Mol. Sci. 23 (9) (2022) 5245, https://doi.org/10.3390/ijms23095245.
[35] M. Montaruli, D. Alberga, F. Ciriaco, D. Trisciuzzi, A.R. Tondo, G.F. Mangiatordi, O. Nicolotti, Accelerating drug discovery by early protein drug target prediction based on a multi-fingerprint similarity search, Molecules 24 (12) (2019) E2233, https://doi.org/10.3390/molecules24122233.
[36] D. Alberga, D. Trisciuzzi, M. Montaruli, F. Leonetti, G.F. Mangiatordi, O. Nicolotti, A new approach for drug target and bioactivity prediction: the multifingerprint similarity search Algorithm (MuSSeL), J. Chem. Inf. Model. 59 (1) (2019) 586–596, https://doi.org/10.1021/acs.jcim.8b00698.
[37] G. Carullo, F. Falbo, A. Ahmed, A. Trezza, B. Gianibbi, O. Nicolotti, G. Campiani, F. Aiello, S. Saponara, F. Fusi, Artificial intelligence-driven identification of morin analogues acting as CaV1.2 channel blockers: synthesis and biological evaluation, Bioorg. Chem. 131 (2023) 106326, https://doi.org/10.1016/j.bioorg.2022.106326.
[38] M.A. Yucel, E. Adal, M.B. Aktekin, C. Hepokur, N. Gambacorta, O. Nicolotti, O. Algul, From deep learning to the discovery of promising VEGFR-2 inhibitors, ChemMedChem 19 (16) (2024) e202400108, https://doi.org/10.1002/cmdc.202400108.
[39] L. Salvadori, M. Paiella, B. Castiglioni, M.L. Belladonna, T. Manenti, C. Ercolani, L. Cornioli, N. Clemente, A. Scircoli, R. Sardella, L. Tensi, A. Astolfi, M.L. Barreca, S. Chiappalupi, G. Gentili, M. Bosetti, G. Sorci, N. Filigheddu, F. Riuzzi, Equisetum Arvense standardized dried extract hinders age-related osteosarcopenia, Biomed. Pharmacother. 174 (2024) 116517, https://doi.org/10.1016/j.biopha.2024.116517.
[40] D. Mendez, A. Gaulton, A.P. Bento, J. Chambers, M. De Veij, E. Félix, M. P. Magariños, J.F. Mosquera, P. Mutowo, M. Nowotka, M. Gordillo-Marañón, F. Hunter, L. Junco, G. Mugumbate, M. Rodriguez-Lopez, F. Atkinson, N. Bosc, C. J. Radoux, A. Segura-Cabrera, A. Hersey, A.R. Leach, ChEMBL: towards direct deposition of bioassay data, Nucleic Acids Res. 47 (D1) (2019) D930–D940, https://doi.org/10.1093/nar/gky1075.
[41] L. Yan, A. Sun, X. Zafirlukast Xu, a Cysteinyl Leukotriene Receptor 1 Antagonist, Reduces the effect of advanced glycation end-products in rat renal mesangial cells in vitro, Med. Sci. Monit. 25 (2019) 8753–8763, https://doi.org/10.12659/MSM.918187.
[42] A. Luginina, A. Gusach, E. Marin, A. Mishin, R. Brouillette, P. Popov, A. Shiriaeva, É. Besserer-Offroy, J.-M. Longpré, E. Lyapina, A. Ishchenko, N. Patel, V. Polovinkin, N. Safronova, A. Bogorodskiy, E. Edelweiss, H. Hu, U. Weierstall, W. Liu, A. Batyuk, V. Gordeliy, G.W. Han, P. Sarret, V. Katritch, V. Borshchevskiy, V. Cherezov, Structure-based mechanism of cysteinyl leukotriene receptor inhibition by antiasthmatic drugs, Sci. Adv. 5 (10) (2019), https://doi.org/10.1126/sciadv.aax2518 eaax2518.
[43] M. Ranieri, G. Tamma, T. Pellegrino, V. Vezzi, C. Ambrosio, C. Grò, A. Di Mise, T. Costa, G. Valenti, S. Cotecchia, Gain-of-Function mutations of the V2 vasopressin receptor in nephrogenic syndrome of inappropriate antidiuresis (NSIAD): a cell-based assay to assess constitutive water reabsorption, Pflügers Archiv 471 (10) (2019) 1291–1304, https://doi.org/10.1007/s00424-019-02307-x.
[44] M. Centrone, I. Saltarella, M. D'Agostino, M. Ranieri, M. Venneri, A. Di Mise, L. Simone, F. Pisani, G. Valenti, M.A. Frassanito, G. Tamma, RhoB plays a central role in hyperosmolarity-induced cell shrinkage in renal cells, J. Cell. Physiol. 239 (9) (2024) e31343, https://doi.org/10.1002/jcp.31343.
[45] M. Ranieri, A. Di Mise, M. Centrone, M. D'Agostino, S.J. Tingskov, M. Venneri, T. Pellegrino, G. Difonzo, F. Caponio, R. Norregaard, G. Valenti, G. Tamma, Olive Leaf Extract (OLE) impaired vasopressin-induced Aquaporin-2 trafficking through the activation of the calcium-sensing receptor, Sci. Rep. 11 (1) (2021) 4537, https://doi.org/10.1038/s41598-021-83850-5.
[46] F. Zhou, C. Ye, X. Ma, W. Yin, T.I. Croll, Q. Zhou, X. He, X. Zhang, D. Yang, P. Wang, H.E. Xu, M.-W. Wang, Y. Jiang, Molecular basis of ligand recognition and activation of human V2 vasopressin receptor, Cell Res. 31 (8) (2021) 929–931, https://doi.org/10.1038/s41422-021-00480-2.
[47] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I. N. Shindyalov, P.E. Bourne, The protein data bank, Nucleic Acids Res. 28 (1) (2000) 235–242, https://doi.org/10.1093/nar/28.1.235.
[48] L. Siragusa, F. Spyrakis, L. Goracci, S. Cross, G. Cruciani, BioGPS: the music for the Chemo- and bioinformatics walzer, Mol Inform 33 (6–7) (2014) 446–453, https://doi.org/10.1002/minf.201400028.
[49] Schrödinger Release 2024-1: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, Impact, Schrödinger, LLC, Schrödinger, LLC, New York, NY, New York, NY; Prime, 2024, 2024.
[50] F. Milletti, L. Storchi, G. Sforna, G. Cruciani, New and original pKa prediction method using grid molecular interaction fields, J. Chem. Inf. Model. 47 (6) (2007) 2172–2181, https://doi.org/10.1021/ci700018y.
[51] B. Dereje, A. Nardos, Dopamine 2 agonists for the management of type 2 diabetes: a systematic review and meta-analysis, J. Diabetes Metab. Disord. 22 (2) (2023) 931–943, https://doi.org/10.1007/s40200-023-01230-4.
[52] A. Pradhan, M. Bhandari, P. Vishwakarma, R. Sethi, Clopidogrel resistance and its relevance: current concepts, J. Fam. Med. Prim. Care 13 (6) (2024) 2187–2199, https://doi.org/10.4103/jfmpc.jfmpc_1473_23.
[53] B.K. Kishore, N.G. Carlson, C.M. Ecelbarger, D.E. Kohan, C.E. Müller, R.D. Nelson, J. Peti-Peterdi, Y. Zhang, Targeting renal purinergic signalling for the treatment of lithium-induced nephrogenic diabetes insipidus, Acta Physiol (Oxf) 214 (2) (2015) 176–188, https://doi.org/10.1111/apha.12507.

[54] J.M. Sands, J.D. Klein, Physiological insights into novel therapies for nephrogenic diabetes insipidus, Am. J. Physiol. Ren. Physiol. 311 (6) (2016) F1149–F1152, https://doi.org/10.1152/ajprenal.00418.2016.
[55] A. Lavergne, C. Vigneau, E. Polard, L. Triquet, N. Rioux-Leclercq, P. Tattevin, L. Golbin, Acute kidney injury during treatment with high-dose cloxacillin: a report of 23 cases and literature review, Int. J. Antimicrob. Agents 52 (3) (2018) 344–349, https://doi.org/10.1016/j.ijantimicag.2018.04.007.
[56] B. Hartung, S. Sampson, S. Leucht, Perphenazine for schizophrenia, Cochrane Database Syst. Rev. 2015 (3) (2015) CD003443, https://doi.org/10.1002/14651858.CD003443.pub3.
[57] R.E. Kast, Adding perphenazine to increase effectiveness of standard glioblastoma chemoirradiation, J BUON 25 (4) (2020) 1676–1686.
[58] J.S. Kelloway, Zafirlukast: the first leukotriene-receptor antagonist approved for the treatment of asthma, Ann. Pharmacother. 31 (9) (1997) 1012–1021, https://doi.org/10.1177/106002809703100912.
[59] S.I. Scorza, S. Milano, I. Saponara, M. Certini, R. De Zio, M.G. Mola, G. Procino, M. Carmosino, F. Moccia, M. Svelto, A. Gerbino, TRPML1-Induced lysosomal Ca2+ signals activate AQP2 translocation and water flux in renal collecting duct cells, Int. J. Mol. Sci. 24 (2) (2023) 1647, https://doi.org/10.3390/ijms24021647.
[60] H.M. Hassan, A. Bagalagel, R. Diri, A. Noor, D. Almasri, D.F. Bannan, M. Z. Nasrullah, M.M.H. Al-Gayyar, Blocking leukotriene receptors improve experimentally induced gastric ulcers in rats by inhibiting inflammation and apoptosis, Int. J. Immunopathol. Pharmacol. 39 (2025), https://doi.org/10.1177/03946320251351083, 3946320251351083.
[61] R.D. Savidge, K.H. Bui, B.K. Birmingham, J.L. Morse, R.C. Spreen, Metabolism and excretion of zafirlukast in dogs, rats, and mice, Drug Metab. Dispos. 26 (11) (1998) 1069–1076.
[62] P.N.R. Dekhuijzen, P.P. Koopmans, Pharmacokinetic profile of zafirlukast, Clin. Pharmacokinet. 41 (2) (2002) 105–114, https://doi.org/10.2165/00003088-200241020-00003.
[63] M. Rubinstein, E. Dvash, Leukotrienes and kidney diseases, Curr. Opin. Nephrol. Hypertens. 27 (1) (2018) 42–48, https://doi.org/10.1097/MNH.0000000000000381.
[64] X. Ye, Y. Dong, J. Han, Y. Huang, M. Wen, R. Zheng, P. Wang, R. Chen, L. Zhao, Repurposing zafirlukast confers renal protection in ischemia-reperfusion injury via suppressing macrophage METosis, Int. Immunopharmacol. 165 (2025) 115459, https://doi.org/10.1016/j.intimp.2025.115459.
[65] J. Demiselle, N. Fage, P. Radermacher, P. Asfar, Vasopressin and its analogues in shock states: a review, Ann. Intensive Care 10 (1) (2020) 9, https://doi.org/10.1186/s13613-020-0628-2.
[66] A.C. Drost, G. Seitz, A. Boehmler, M. Funk, K.P. Norz, A. Zipfel, X. Xue, L. Kanz, R. Möhle, The G protein-coupled receptor CysLT1 mediates chemokine-like effects and prolongs survival in chronic lymphocytic leukemia, Leuk. Lymphoma 53 (4) (2012) 665–673, https://doi.org/10.3109/10428194.2011.625578.
[67] M. Matsuyama, K. Funao, J. Chargui, J.-L. Touraine, T. Nakatani, R. Yoshimura, United States transplantation proceedings, the role of Cysteinyl-LT(1)Receptor (CysLT(1)R), Renal Ischemia-Reperfusion Injury 41 (1) (2009-01) 73.