Aspects of Molecular Medicine 3 (2024) 100036

Contents lists available at ScienceDirect

Aspects of Molecular Medicine

journal homepage: www.journals.elsevier.com/aspects-of-molecular-medicine

Investigating the potential of natural compounds and known drugs against obstructive airway inflammation in inhibiting TRPC6

Uzma Saqib [a,1], Isaac S. Demaree [b,1], Alexander G. Obukhov [b,c], Krishnan Hajela [a,*]

[a] School of Life Sciences, Devi Ahilya Vishwavidyalaya, Vigyan Bhawan, Khandwa Road Campus, Indore, 452 001, MP, India
[b] Department of Anatomy, Cell Biology & Physiology, Indiana University School of Medicine, Indianapolis, IN, 46202, USA
[c] Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, IN, 46202, USA

ARTICLE INFO

Handling Editor: Prof A Angelo Azzi

ABSTRACT

The Transient Receptor Potential Canonical 6 (TRPC6) channel was implicated in the pathogenesis of pulmonary airway inflammation, chronic obstructive pulmonary disease, pulmonary edema, and pulmonary fibrosis. It was also proposed that Ca^{2+} influx through TRPC6 may contribute to triggering pulmonary inflammatory responses. The ChEMBL database lists 44 drugs that are clinically used to treat asthma, a type of obstructive airway inflammation (OAI). Since the mode of action and targets are not fully elucidated for many of these 44 drugs, we used computational approaches to determine the drugs' potential to interact with the inhibitor binding site on the TRPC6 protein. We also screened a library of natural compounds to retrieve the phytochemicals with a potential to interact with TRPC6. The binding affinities of two well-known TRPC6 inhibitors, BTDM and 2-aminoethoxydiphenyl borate (2-APB), were compared with those of the screened compounds. We found that despite stable *in silico* binding and a well-defined three-dimensional molecular interaction pattern with the TRPC6 protein of the two top-scoring compounds, montelukast and solanesol, the molecules from the approved-drug and natural-compound libraries respectively, failed to show any significant efficacy in the *in vitro* assays.

1. Introduction

With increasing industrialization and automobile usage, there has been a several fold increase in cases of airway inflammation which leads to the respiratory diseases, including asthma and chronic obstructive pulmonary disease (COPD) (Lee et al., 2021). The major pollutants, such as ozone (O_3), asbestos fibers, smoke from various sources, etc. are known to promote reactive oxygen species (ROS) generation in the lung tissues leading to oxidative stress (Sierra-Vargas et al., 2023). The transient receptor potential canonical 6 channel (TRPC6) is highly expressed in the lungs (Hofmann et al., 1999), and its gene is the sixth most highly expressed gene in the lungs of patients presenting with COPD (Dhong et al., 2023), suggesting that TRPC6 may play a role in the pathophysiology of some lung disease (Dietrich et al., 2017; Saqib et al., 2023).

TRPC6 is a redox-regulated Ca^{2+} permeable cation channel, and its involvement in airway inflammation is well documented (Williams and Roman, 2015). Chen et al. showed that genetic ablation or pharmacological inhibition of TRPC6 protected mice from O_3-induced airway inflammatory responses (Chen et al., 2020). Consistently, the role of TRPC6 in airway inflammation was supported by the observation that TRPC6 knockout mice exhibited an attenuated O_3-induced neutrophil recruitment to airway epithelial cells in part due to decreased intercellular adhesion molecule-1 (ICAM-1) expression (Chen et al., 2019). Furthermore, TRPC6 knockout mice exhibited reduced allergic responses to ovalbumin sensitization which were accompanied by a decreased airway eosinophilia, lower blood IgE levels, and reduced proinflammatory IL-5 and IL-13 cytokine concentrations in the bronchoalveolar lavage (Sel et al., 2008). As we noted above, TRPC6 is one of the most highly expressed genes in COPD patients. Specifically, the upregulation of TRPC6 was verified in particulate matter (PM)-stimulated macrophages from COPD samples affirming the channel's role in OAI (Dhong et al., 2023).

According to a study by Hong et al., calcium entry via TRPC6 channels plays a critical role in the regulation of airway smooth muscle cell proliferation and airway remodeling (Wang et al., 2017). It was proposed that oxidative stress-induced Ca^{2+} influx via TRPC6 is the main triggering event responsible for diseases associated with airway

* Corresponding author.
E-mail address: hajelak@gmail.com (K. Hajela).
[1] Contributed equally.

https://doi.org/10.1016/j.amolm.2024.100036
Received 4 November 2023; Received in revised form 11 February 2024; Accepted 13 February 2024
Available online 15 February 2024

inflammation (Chen et al., 2020). Hence, targeting TRPC6 during the initial phase of the inflammatory cascade is an attractive strategy to combat the complications of COPD.

Since TRPC6 is a critical player of OAI associated diseases, we screened a library of compounds from the ChEMBL database (Gaulton et al., 2011), searching for compounds that have been approved for OAI. This led us to retrieve 44 drugs approved for OAI and associated diseases. We hypothesized that drugs effective against OAI might be binding and potentially inhibiting TRPC6. In parallel, we also screened a database of natural compounds with known pharmaceutical potency against OAI in order to identify any potential molecule capable of interacting with TRPC6. To validate our standard docking protocol, we docked two well-known TRPC6 inhibitors, BTDM and 2-aminoethoxydiphenyl borate (2-APB). Several molecules were identified with a potential to modulate TRPC6 activity. However, the overall study resulted in an inconclusive correlation between docking results and the *in vitro* assay, as the high scoring compounds from both libraries do not show any significant efficacy against TRPC6.

2. Materials and methods

2.1. Docking studies

The three-dimensional structure of TRPC6 (pdb: 7dxf) was retrieved from the Protein Data Bank. It was truncated to retain the A chain. The bound TRPC6 inhibitor BTDM was extracted from the structure and incorporated in the docking library. Other potent TRPC6 inhibitors including 2-APB and larixyl acetate were also added to the docking database. Further, the ChEMBL database was explored for *"approved drugs"* against "*Obstructive airway inflammation"* (OAI) which returned a total of *44 compounds*. These compounds are approved drugs against OAI and associated diseases. Another nature compound database, the puretitre_200 compound library (Caithness Biotechnologies - Focused libraries for drug discovery), was retrieved from Caithness Biotechnologies comprising a collection of 200 pure natural compounds. We next specified the binding site coordinates in TRPC6 as X = 126.4, Y = 150.4, Z = 158.8 for the incoming compounds for docking. e-LEA3D (Douguet et al., 2005), a computational-aided drug design web server, was utilized to dock the compounds from the two compound libraries along with the two well-known TRPC6 inhibitors. In this program, the user first builds a scoring function and then either uploads a library of molecules or selects the de novo drug design option. In our case, we specified our own libraries. The results were analyzed for their dock scores. These dock scores are calculated by using the ChemPLP scoring function implemented in PLANTS (Korb et al., 2009). *N*egative scores indicate favourable binding. The **more negative score suggests** a potentially stronger binding. On the other hand, the percentage is a score relative to the function (for example: 90% for docking + 10% for the property molecular weight) and helps to rank compounds. The property molecular weight refers to the molecular weight of the drug which influences its binding to the target.The final conformations were visualized in Chimera (Pettersen et al., 2004) and Discovery studio software (BIOVIA Product Portfolio – BIOVIA – Dassault Systèmes® (3ds.com).

2.2. Cell culture and transfection

HEK cells were purchased at American Type Culture Collection (Manassas, VA, USA). Eagle's minimum essential medium supplemented with 10% fetal bovine serum was used to culture the HEK cells. Cells were co-transfected with the human TRPC6 cDNA and the histamine H1 receptor cDNA using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA) as described in (Chen et al., 2017). After transfection, cells were split and plated on 25 mm glass coverslips and cultured for an additional 30–48 h before electrophysiological recordings.

Table 1
The percent and docking scores of the best poses for all 5 compounds.

Compound	Score (%)	Dock Score (kcal/mol)
Montelukast	65.85	−98.77
Solanesol	74.59	−111.88
BTDM	44.70	−67.05
2-ABP	45.7	−68.45
Larixyl acetate	43.73	−72.82

2.3. Electrophysiological recordings

Electrophysiological experiments were performed using the whole-cell voltage-clamp technique as described previously (Chen et al., 2017). An Axopatch 200B amplifier and Digidata 1400 digitizer (Molecular Devices, San Jose, CA, USA) were used to measure the histamine-induced currents in TRPC6-HEK cells. Series resistance compensation was set to 50–70%. The holding potential was set to −60 mV. Then, 150 ms voltage ramps from −100 to +100 mV were applied every 2 s. The currents were filtered at 3 kHz. The extracellular solution contained (in mM) 145 NaCl, 2.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 5.5 glucose (pH 7.2 adjusted with NaOH). The pipette solution contained (in mM) 125 $CsMeSO_3$, 2 $MgCl_2$, 3.8 $CaCl_2$, 10 EGTA, and 10 HEPES (pH 7.2 adjusted with Trizma base). No leak subtraction was done. Cells with leak currents greater than 100 pA were not included in the statistical analysis. pCLAMP 10 software was used for data acquisition and analyses. The experiments were performed at 22–25 °C.

2.4. Statistical analysis

The statistical analysis was performed using Excel 365 and SigmaPlot 12.5 software. The curves were fit to a second order polynomial function using Excel to determine the rate of decay of current independent of the applied compound. The theoretical value was compared to the actual measured value, and the decrease in magnitude from the theoretical to the experimental values were calculated as a percent of decrease. To determine whether there was a statistically significant difference between the tested groups, we used the Kruskal–Wallis one-way ANOVA on ranks test, followed by the Dunn's post hoc multiple comparisons versus control group test. The significance level was set to $P < 0.05$.

3. Results

3.1. Molecular docking

In this study, we used two libraries: the ChEMBL compound library and the Puretitre compound library. We first searched the ChEMBL library to identify the "approved" drugs against "Obstructive airway inflammation" and retrieved 44 compounds that were later computationally docked to the TRPC6 protein. The Puretitre compound library is a natural compound library with about 200 compounds isolated from diverse phytoextracts. All those 200 compounds were computationally docked to the TRPC6 protein. Three known, potent TRPC6 inhibitors (BTDM,2-APB, and larixyl acetate) were also docked to the TRPC6 protein in a similar fashion as the other selected compounds from the two libraries. The redocking of BTDM to the TRPC6 protein was done to validate our standard docking protocol and to confirm that the program docks the compound to its original binding site on TRPC6 and in the original conformation. e-LEA3D, a computational-aided drug design web server, has been utilized to perform the docking studies because of its versatility with various small molecules and the ease of use. While docking the compounds, we determining the control inhibitors' and experimental compounds' *in silico* binding efficacy and docked conformations and then compared the obtained values. We found that montelukast from the ChEMBL database and solanesol from the Puretitre library were the highest scoring compounds with a dock score of −98.77

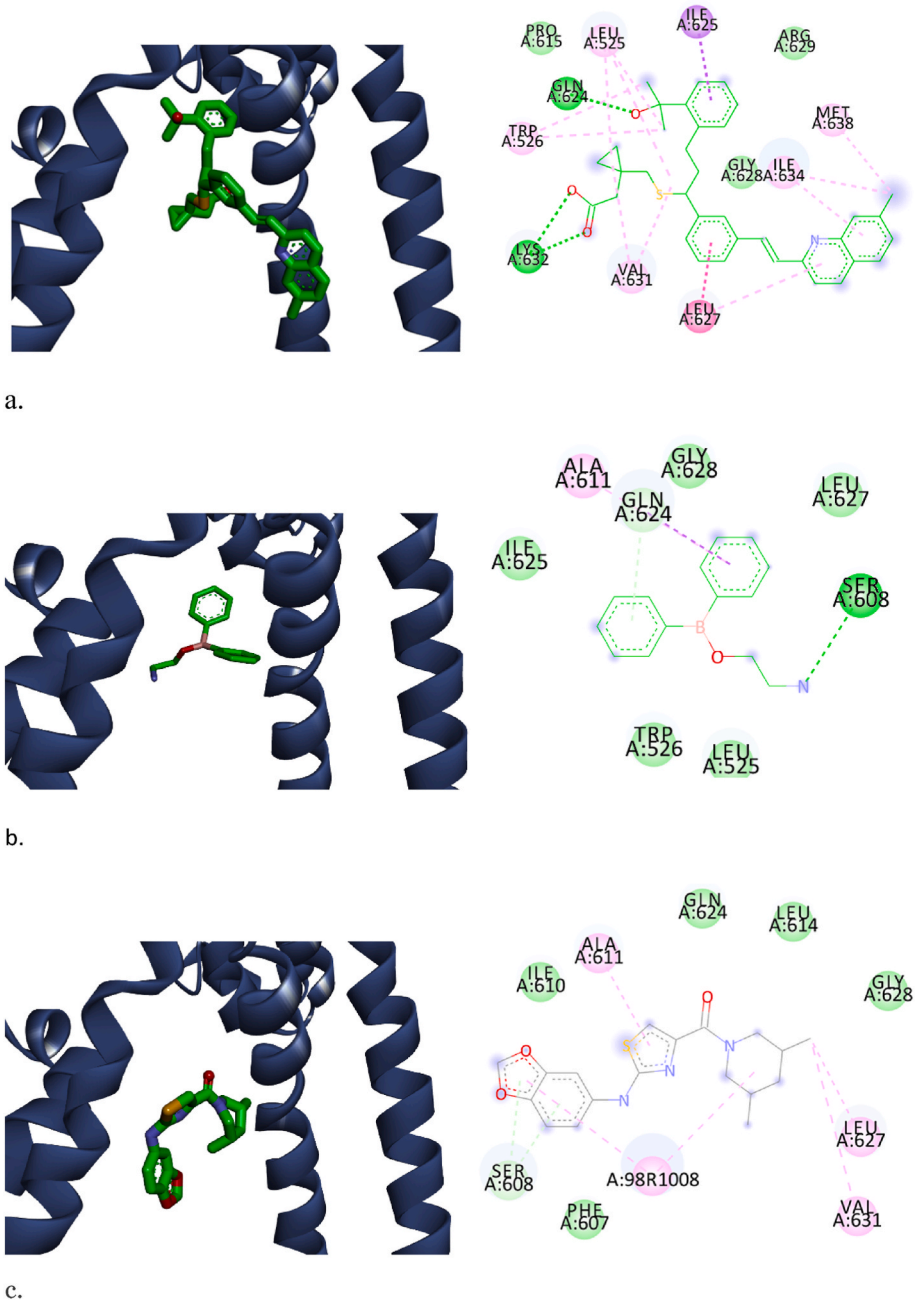
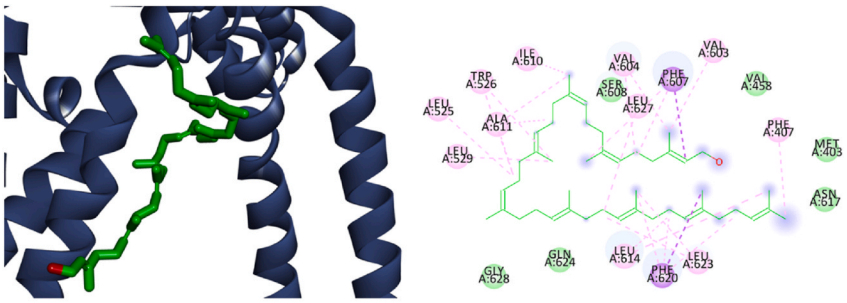


Fig. 1. Docked complexes of TRPC6 with (a) montelukast (b) 2-ABP; (c) BTDM; (d) solanesol; (e) larixyl acetate (f) all of these. The left panel represent three-dimensional receptor-inhibitor interactions while the right panel shows two-dimensional representations of various molecular interactions between TRPC6 and inhibitors including H-bond (dark green), Van der Waals (light green), Pi Alkyl (pink), Pi sigma (purple). TRPC6 is colored in blue ribbons, while respective inhibitors are shown as atom-colored sticks.

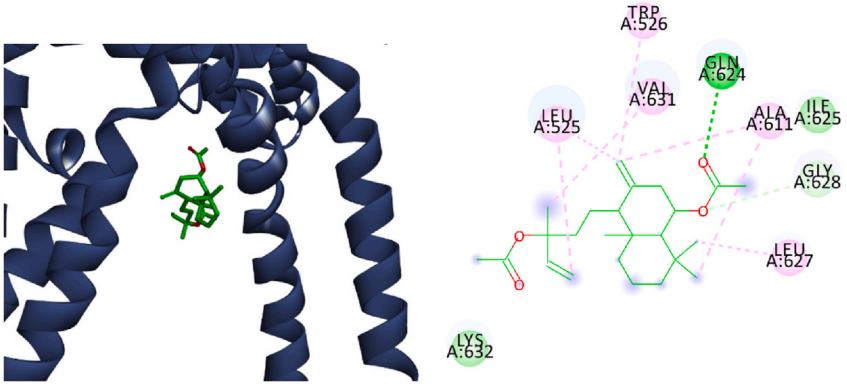
kcal/mol and −111.88 kcal/mol, respectively (Table 1). On the other hand, the dock scores of BTDM, 2-ABP, and larixyl acetate were −67.05 kcal/mol, −68.45 kcal/mol, and −72.82 kcal/mol, respectively. Table 1 refers to the percent and dock scores of known TRPC6 inhibitors along with montelukast and solanesol. We determined the binding conformations of each of these compounds in the TRPC6 binding site (Fig. 1). Fig. 1a shows that montelukast docks well between the channel's pore and voltage-sensor-like domain (VSLD), suggesting that the compound may be capable of modulating TRPC6 activity. Remarkably, montelukast makes several Hydrogen bond interactions including those with TRPC6's Lys632 and Gln624 as well as many hydrophobic interactions with TRPC6's Trp526, Leu627, Leu525, Val631, etc. We found that the conformations and three-dimensional interactions of 2-ABP (Fig. 1b), BTDM (Fig. 1c), solanesol (Fig. 1d), and larixyl acetate (Fig. 1e) were similar to montelukast (Fig. 1a). This may suggest a similar mode of interaction and binding of all five compounds. Tables 2–6 display the detailed information of molecular interactions of these compounds including the distance and the types of non-covalent interactions.

3.2. *Electrophysiological in vitro assay*

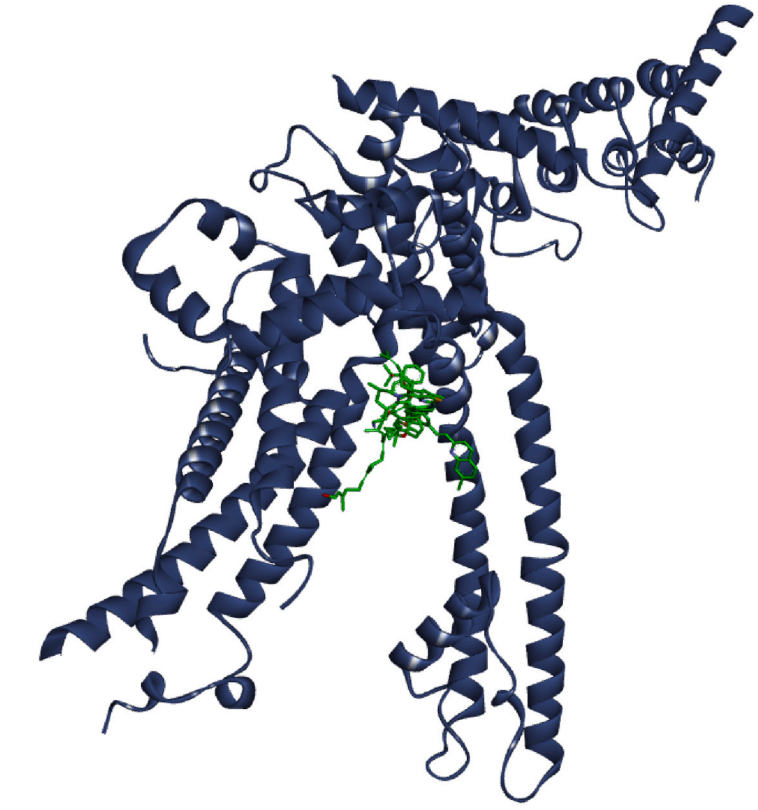
To test the inhibitory potential of the two identified compounds (montelukast and solanesol), we used the patch-clamp whole-cell approach. We determined the effects of the two highest scoring



d.



e.



f.

Fig. 1. (*continued*).

Table 2
List of molecular interactions between TRPC6 and Montleucast. The residues are numbered by their location and Chain. The compound is named as MOL1.

Name	Distance	Category
A:GLN624:HE21 -:MOL1:O	1.92096	Hydrogen Bond
A:LYS632:HZ1 -:MOL1:O	1.99161	Hydrogen Bond
A:LEU627:C,O; GLY628:N -:MOL1	4.11712	Hydrophobic
A:LEU525 -:MOL1	5.44544	Hydrophobic
A:VAL631 -:MOL1	5.36874	Hydrophobic
A:VAL631 -:MOL1	4.6943	Hydrophobic
:MOL1 - A:LEU525	4.22805	Hydrophobic
:MOL1:CL - A:ILE634	4.39282	Hydrophobic
:MOL1:CL - A:MET638	5.48736	Hydrophobic
:MOL1:C - A:LEU525	4.81796	Hydrophobic
A:TRP526 -:MOL1:C	4.50682	Hydrophobic
A:TRP526 -:MOL1:C	4.94244	Hydrophobic
A:TRP526 -:MOL1:C	4.64563	Hydrophobic
:MOL1 - A:LEU627	5.29997	Hydrophobic
:MOL1 - A:ILE634	4.78271	Hydrophobic
:MOL1 - A:ILE625	4.07382	Hydrophobic

Table 3
List of molecular interactions between TRPC6 and BTDM. The residues are numbered by their location and Chain, whereas the compound is named as MOL1.

Name	Distance	Category
:MOL1:H -:MOL1:N	2.25002	Hydrogen Bond
:MOL1 - A:PHE620	4.31987	Hydrophobic
A:ILE610 -:MOL1	4.63736	Hydrophobic
A:LEU614 -:MOL1	3.95266	Hydrophobic
:MOL1:C - A:ILE610	4.31902	Hydrophobic
:MOL1:C - A:ILE610	4.56888	Hydrophobic
:MOL1:C - A:LEU614	4.59108	Hydrophobic
:MOL1 - A:LEU627	5.11436	Hydrophobic
:MOL1 - A:LEU614	5.46459	Hydrophobic
:MOL1 - A:LEU614	5.48224	Hydrophobic

Table 4
List of molecular interactions between TRPC6 and larixyl acetate. The residues are numbered by their location and Chain. The compound is named as MOL1.

Name	Distance	Category
A:LYS632:HZ1 -:mol1:O	2.34466	Hydrogen Bond
A:LYS632:HZ3 -:mol1:O	2.60487	Hydrogen Bond
A:ALA611 -:mol1	5.19926	Hydrophobic
A:ALA611 -:mol1:C	3.85985	Hydrophobic
:mol1:C - A:VAL631	3.78762	Hydrophobic
:mol1:C - A:LYS632	4.41907	Hydrophobic
:mol1:C - A:LEU525	4.80142	Hydrophobic
:mol1:C - A:VAL631	3.92973	Hydrophobic

compounds on histamine (10 μM)-induced inward and outward currents through the homomeric TRPC6 channels expressed in HEK cells. We found that 20 μM montelukast only slightly decreased histamine-induced TRPC6 currents (15.8 ± 4.0%) compared to a more potent effect of larixyl acetate (57.9 ± 16.4%, Fig. 2), a known inhibitor of TRPC6. The solanesol effect on histamine-induced currents was significantly weaker (10.9 ± 4.3%) compared to larixyl acetate-mediated inhibition of TRPC6 currents ($p = 0.018$). These data suggest that solanesol and montelukast do not significantly affect TRPC6 function in the HEK cell expression model. We not only wanted to know the differences between the percent of inhibition but also sought to quantify each inhibitor's activity. A paired T-test was performed to compare current between larixyl acetate (−1.62 ± 2.03 pA/pF) and histamine alone (−2.79 ± 2.48 pA/pF), and as expected, the current was found to be significantly inhibited by larixyl acetate compared to histamine alone ($p = 0.048$). The same analysis was done for the current in the presence of solanesol, and although it showed significantly less inhibition than

Table 5
List of molecular interactions between TRPC6 and Solenesol. The residues are numbered by their location and Chain. The compound is named as MOL1.

Name	Distance	Category
A:GLN624:HE21 -:MOL1:O	1.92096	Hydrogen Bond
A:LYS632:HZ1 -:MOL1:O	1.99161	Hydrogen Bond
A:LYS632:HZ2 -:MOL1:O	2.59844	Hydrogen Bond
A:LEU627:C,O; GLY628:N -:MOL1	4.11712	Hydrophobic
A:LEU525 -:MOL1	5.44544	Hydrophobic
A:VAL631 -:MOL1	5.36874	Hydrophobic
A:VAL631 -:MOL1	4.6943	Hydrophobic
:MOL1 - A:LEU525	4.22805	Hydrophobic
:MOL1:CL - A:ILE634	4.39282	Hydrophobic
:MOL1:CL - A:MET638	5.48736	Hydrophobic
:MOL1:C - A:LEU525	4.81796	Hydrophobic
A:TRP526 -:MOL1:C	4.50682	Hydrophobic
A:TRP526 -:MOL1:C	4.94244	Hydrophobic
A:TRP526 -:MOL1:C	4.64563	Hydrophobic
:MOL1 - A:LEU627	5.29997	Hydrophobic
:MOL1 - A:ILE634	4.78271	Hydrophobic
:MOL1 - A:ILE625	4.07382	Hydrophobic

Table 6
List of molecular interactions between TRPC6 and 2-ABP. The residues are numbered by their location and Chain. The compound is named as MOL1.

Name	Category	Name
A:GLN624:NE2 -:MOL1:O	2.73085	Hydrogen Bond
A:LYS632:NZ -:MOL1:O	2.84483	Hydrogen Bond
A:LYS632:NZ -:MOL1:O	2.87918	Hydrogen Bond
A:ILE625:CD1 -:MOL1	3.51828	Hydrophobic
A:LEU627:C,O; GLY628:N -:MOL1	4.11712	Hydrophobic
A:LEU525 -:MOL1	5.44544	Hydrophobic
A:VAL631 -:MOL1	5.36874	Hydrophobic
A:VAL631 -:MOL1	4.6943	Hydrophobic
:MOL1 - A:LEU525	4.22805	Hydrophobic
:MOL1:CL - A:ILE634	4.39282	Hydrophobic
:MOL1:CL - A:MET638	5.48736	Hydrophobic
:MOL1:C - A:LEU525	4.81796	Hydrophobic
A:TRP526 -:MOL1:C	4.50682	Hydrophobic
A:TRP526 -:MOL1:C	4.94244	Hydrophobic
A:TRP526 -:MOL1:C	4.64563	Hydrophobic
:MOL1 - A:LEU627	5.29997	Hydrophobic
:MOL1 - A:ILE634	4.78271	Hydrophobic

larixyl acetate, the difference between current densities in the presence of solanesol + histamine (−4.61 ± 3.62 pA/pF) and histamine alone (−4.96 ± 3.72 pA/pF) was also significant ($p = 0.018$). This suggests that although it is less potent than larixyl acetate, solanesol can also inhibit TRPC6 currents as the dock score suggested. However, there was no significant inhibition of the histamine-induced currents (−7.27 ± 7.08 pA/pF) when montelukast (−5.86 ± 6.13 pA/pF) was added ($p = 0.063$) to the bath. When analyzing the montelukast data, we had to use the paired Wilcoxon Signed Rank Test because the data were not normally distributed. This data indicates that montelukast may have no inhibitory action on TRPC6, unlike solanesol and larixyl acetate.

4. Discussion

Molecular Docking is a powerful tool for investigating protein-ligand interactions. Here we docked the compounds from two natural and approved drug OAI databases to the TRPC6 protein in order to search for the potential modulators of TRPC6 activity. By using the ChEMBL library, we planned to establish whether the already approved drugs against OAI can also modulate TRPC6 function. Our working hypothesis was based on the reports that TRPC6 activity contributes to OAI pathogenesis (Dhong et al., 2023). Indeed, TRPC6 is an important mediator of Ca^{2+} influx in pulmonary tissues of OAI patients, and the channels may be directly involved in the development of OAI. Therefore, drugs relieving OAI may potentially target TRPC6 as well. Thus, we thought

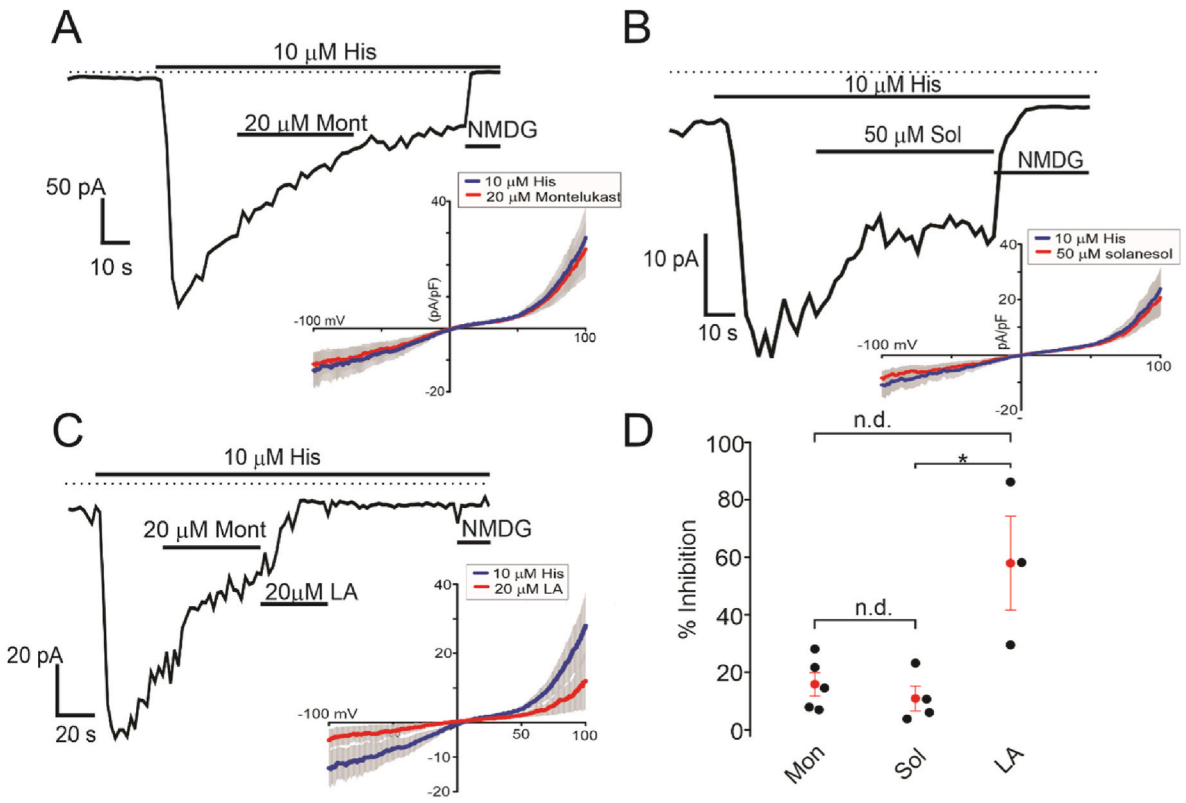


Fig. 2. Comparison of histamine-induced current amplitudes in HEK cells expressing TRPC6 and histamine H1 receptor proteins in the presence of the tested compounds. A, B, C, time course of currents activated by histamine (10 μM) in the presence of histamine or the mixture of histamine and either montelukast (Mon), solanesol (Sol), larixyl acetate (LA), or $NMDG^{+}$-0Ca as indicated by the horizontal bars. Dotted lines indicate the level of zero current. The insets show the current-voltage relationships of TRPC channels recorded during voltage ramps from −100 mV to 100 mV. Blue curves represent the average current in the presence of 10 μM Histamine. Red curves show the average current in the presence of the indicated compound and 10 μM Histamine. D, Comparison of the inhibitory effect of montelukast (Mon), solanesol (Sol), and larixyl acetate (LA) on histamine induced TRPC6 current in HEK cells at −60 mV. The red dots represent the means of each set, and the error bars show the standard error of mean (SEM). The black dots represent the individual data points for each tested group. One Way Analysis of Variance with Dunn's post hoc test was used to analyze the data.

that some of OAI drugs may serve as TRPC6 inhibitors. The second library of natural compounds was searched to retrieve potent phytochemicals having the ability to dock and potentially modulate TRPC6 activity with a comparable or greater affinity as compared to the known TRPC6 inhibitors (BTDM and 2-APB).

We did identify two top scoring compounds, montelukast and solanesol, which were docking to the TRPC6 protein with considerably higher docking scores as compared to the well-known TRPC6 inhibitors. The computational data suggested that these two compounds may theoretically modulate TRPC6 function. However, we later found that both montelukast and solanesol either failed or showed a very small inhibitory effect on TRPC6 activity in the patch-clamp whole cell assay, using the HEK cell heterologous expression model.

5. Conclusions

Although molecular docking is widely used to identify potential ligand-protein interactions at the atomic level and is very effective in gaining insight into the mechanisms governing the formation of the ligand-protein complexes, the computational static docking results should be confirmed at least with molecular dynamics simulations. Furthermore, any computational data should be always validated in the relevant *in vitro* assays before a conclusion about ligand efficacy can be drawn. The failure of montelukast and solanesol, both exhibiting higher docking scores than known TRPC6 inhibitors, to show a greater inhibitory potential against TRPC6 in a functional assay is an example demonstrating the limitation of sole molecular docking approach.

CRediT authorship contribution statement

Uzma Saqib: Conceptualization, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Isaac S. Demaree:** Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Alexander G. Obukhov:** Data curation, Formal analysis, Investigation, Project administration, Resources, Supervision, Writing – review & editing. **Krishnan Hajela:** Project administration, Writing – review & editing, Conceptualization, Supervision, Writing – original draft.

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.

Acknowledgements

The authors thank the Department of Life Science, DAVV for facilities. Financial assistance from UGC-DSKPDF (BL/20–21/0482 (S-90)) to Dr. Uzma Saqib is gratefully acknowledged.

References

Chen, X., Li, W., Riley, A.M., Soliman, M., Chakraborty, S., Stamatkin, C.W., Obukhov, A. G., 2017. Molecular determinants of the sensitivity to Gq/11-Phospholipase C-dependent gating, Gd3+ potentiation, and Ca2+ permeability in the transient receptor potential canonical type 5 (TRPC5) channel. J. Biol. Chem. 292, 898–911.

Chen, Q.-Z., Zhou, Y.-B., Zhou, L.-F., Fu, Z.-D., Wu, Y.-S., Chen, Y., Li, S.-N., Huang, J.-R., Li, J.-H., 2019. TRPC6 modulates adhesion of neutrophils to airway epithelial cells via NF-κB activation and ICAM-1 expression with ozone exposure. Exp. Cell Res. 377, 56–66.

Chen, Q., Zhou, Y., Zhou, L., Fu, Z., Yang, C., Zhao, L., Li, S., Chen, Y., Wu, Y., Ling, Z., Wang, Y., Huang, J., Li, J., 2020. TRPC6-dependent Ca2+ signaling mediates airway inflammation in response to oxidative stress via ERK pathway. Cell Death Dis. 11.

Dhong, K., Lee, J., Yoon, Y., Park, H., 2023. Identification of TRPC6 as a novel diagnostic biomarker of PM-Induced Chronic Obstructive Pulmonary Disease using machine learning models. Genes 14 (2), 284.

Dietrich, A., Steinritz, D., Gudermann, T., 2017. Transient receptor potential (TRP) channels as molecular targets in lung toxicology and associated diseases. Cell Calcium 67, 123–137.

Douguet, D., Munier-Lehmann, H., Labesse, G., Pochet, S., 2005. LEA3D: a computer-aided ligand design for structure-based drug design. J. Med. Chem. 48, 2457–2468.

Gaulton, A., Bellis, L.J., Bento, A.P., Chambers, J., Davies, M., Hersey, A., Light, Y., McGlinchey, S., Michalovich, D., Al-Lazikani, B., Overington, J.P., 2011. ChEMBL: a large-scale bioactivity database for drug discovery. Nucleic Acids Res. 40, D1100–D1107.

Hofmann, T., Obukhov, A.G., Schaefer, M., Harteneck, C., Gudermann, T., Schultz, G., 1999. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. Nature 397, 259–263.

Korb, O., Stützle, T., Exner, T.E., 2009. Empirical scoring functions for advanced protein-ligand docking with PLANTS. J. Chem. Inf. Model. 49 (1), 84–96.

Lee, Y.-G., Lee, P.-H., Choi, S.-M., An, M.-H., Jang, A.-S., 2021. Effects of air pollutants on airway diseases. Int. J. Environ. Res. Publ. Health 18, 9905.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera–A visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.

Saqib, U., Munjuluri, S., Sarkar, S., Biswas, S., Mukherjee, O., Satsangi, H., Baig, M.S., Obukhov, A.G., Hajela, K., 2023. Transient receptor Potential Canonical 6 (TRPC6) channel in the Pathogenesis of diseases: a jack of many trades. Inflammation 46 (4), 1144–1160.

Sel, S., Rost, B.R., Yildirim, A.Ö., Sel, B., Kalwa, H., Fehrenbach, H., Renz, H., Gudermann, T., Dietrich, A., 2008. Loss of classical transient receptor potential 6 channel reduces allergic airway response. Clin. Exp. Allergy 38 (9), 1548–1558.

Sierra-Vargas, M.P., Montero-Vargas, J.M., Debray-García, Y., Vizuet-de-Rueda, J.C., Loaeza-Román, A., Terán, L.M., 2023. Oxidative stress and air pollution: its impact on chronic respiratory diseases. Int. J. Mol. Sci. 24, 853.

Wang, H., Peng, G., Hao, B., Liao, B., Zhao, Z., Zhou, Y., Peng, F., Ye, X., Huang, L., Zheng, M., Pu, J., Liang, C., Yi, E., Peng, H., Li, B., Ran, P., 2017. Nicotine-induced airway smooth muscle cell proliferation involves TRPC6-dependent calcium influx via α7 nAChR. Cell. Physiol. Biochem. 43 (3), 986–1002.

Williams, K., Roman, J., 2015. Studying human respiratory disease in animals - role of induced and naturally occurring models. J. Pathol. 238, 220–232.