УДК 616.357:577.175.53:577.2
https://doi.org/10.20538/1682-0363-2024-4-136-144

Studying molecular interactions of synthetic glucocorticoids with TRPM8 by molecular docking

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ABSTRACT

Aim. To carry out *in silico* screening of interactions of synthetic glucocorticoids with TRPM8.

Materials and methods. Information on the structure of the ligands was obtained from the PubChem chemical database in sdf format. The TRPM8 protein model was downloaded from the AlphaFold Protein Structure Database (AlpahaFold ID: AF-Q7Z2QW). Prediction of molecular cavities and coordinates of their centers was carried out on the PrankWeb web server. Modeling of molecular interactions was carried out using AutoDock (generation of 100 epochs) and MOE (generation of 300 poses) software.

Results. The study revealed that the ligands formed stable complexes with TRPM8, but all of them, except for beclomethasone dipropionate, did not interact with the Tyr745 amino acid residue (the key binding site for channel activation). Thus, it can be assumed that glucocorticoids are most likely inhibitors of this ion channel. Of all glucocorticoids, special attention was paid to prednisolone, flunisolide, and budesonide, since the results of molecular docking of these molecules using AutoDock and MOE showed comparable data.

Conclusion. The results obtained provide an insight into the therapeutic potential of these drugs in terms of their use in the treatment of cold-induced airway hyperresponsiveness and also expand the potential for their personalized use in the treatment of bronchial asthma and COPD.

Keywords: TRPM8, molecular docking, glucocorticoids, *in silico*

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

For citation: Timkin P.D., Kotelnikov D.D., Timofeev E.A., Naumov D.E., Borodin E.A Studying molecular interactions of synthetic glucocorticoids with TRPM8 by molecular docking. *Bulletin of Siberian Medicine.* 2024;23(4):136–144. https://doi.org/10.20538/1682-0363-2024-4-136-144.

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Исследование молекулярных взаимодействий синтетических глюкокортикоидов с TRPM8 методом молекулярного докинга

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РЕЗЮМЕ

Цель: осуществление *in silico* скрининга взаимодействий синтетических глюкокортикоидов с TRPM8.

Материалы и методы. Информация о структуре лигандов была получена из базы данных химических соединений PubChem в sdf-формате. Модель белка TRPM8 загружена из базы данных AlphaFold Protein Structure Database (AlpahaFold ID: AF-Q7Z2QW). Предсказание молекулярных полостей и координат их центров осуществлялось на веб-сервере PrankWeb. Моделирование молекулярного взаимодействия проводили с использованием двух программ: AutoDock (генерация 100 эпох) и MOE (генерация 300 поз).

Результаты. В ходе проведения исследования выяснилось, что лиганды образуют стабильные комплексы с TRPM8, но при этом все, кроме беклометазона дипропионата, не взаимодействуют с аминокислотным остатком Tyr745 (ключевой сайт связывания для активации канала). Таким образом, можно полагать, что глюкокортикоиды, вероятнее всего, являются ингибиторами данного ионного канала. Из всех глюкокортикоидов особое внимание было уделено преднизолону, флунизолиду и будесониду, так как результаты молекулярного докинга этих молекул с использованием AutoDock и MOE демонстрируют сопоставимые данные.

Заключение. Полученные результаты позволяют взглянуть на терапевтический потенциал данных препаратов в аспекте их использования при лечении холод-индуцированной гиперреактивности дыхательных путей, а также расширяют потенциал их персонализированного применения в терапии бронхиальной астмы и хронической обструктивной болезни легких.

Ключевые слова: TRPM8, молекулярный докинг, глюкокортикоиды, *in silico*

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Для цитирования: Тимкин П.Д., Котельников Д.Д., Тимофеев Э.А., Наумов Д.Е., Бородин Е.А. Исследование молекулярных взаимодействий синтетических глюкокортикоидов с trpm8 методом молекулярного докинга. *Бюллетень сибирской медицины.* 2024;23(4):136–144. https://doi.org/10.20538/1682-0363-2024-4-136-144.

INTRODUCTION

TRPM8 is an ion channel that provides Ca^{2+} and Na^{+} supply into the cell. This channel is a homotetramer, each subunit of which contains six transmembrane domains (S1–S6) [1].

This channel is known to play an essential role in the sensation of cold. Activation of the channel occurs at certain temperatures (10–28 °C) or under the influence of chemical agents (for example, menthol, icilin) [2-4]. Due to its functional role, TRPM8 is expressed in a subpopulation of primary afferent neurons that innervate cold-hypersensitive tissues, including the skin, oral epithelium, teeth, nasal mucosa, tongue, and cornea. There is also evidence of the presence of this channel in the epithelium of

lung tissue and on leukocytes, including those not in contact with the external environment, which implies the presence of endogenous modulators of TRPM8 activity. The activity of the ion channel is combined with the transcriptional regulation of important immunomodulatory agents interleukin (IL)-6 and IL-8, which are often expressed during inflammation in the respiratory tract [5].

Commonly used drugs to relieve bronchial asthma are synthetic glucocorticoids (GCs), which have an anti-inflammatory effect. The main mechanism of action of GCs is mediated by binding to the cytosolic glucocorticoid receptor. After this, the newly formed complex, which has undergone dimerization, is translocated into the cell nucleus, resulting in the regulation of gene expression. This process is usually called transcriptional activation or transactivation [6, 7].

It is generally accepted that GC hormones do not bind to ion channels of the TRP family, at least there are no experimental data demonstrating this. However, there is evidence of modulation of TRP receptors by some steroid hormones, such as testosterone, estradiol, and androgens [8]. In our previous studies on the search for potential ligands for TRPM8 using *in silico* methods with neural networks, we found that the synthetic GC dexamethasone is a candidate for interaction with the receptor. Data from rigid molecular docking in the region close to amino acid residue Tyr745 demonstrated the hypothetical possibility of complex formation [9]. The Tyr745 residue is the most important in the implementation of the TRPM8 function, since in the native state, it is this residue that forms a hydrogen bond with menthol, resulting in activation of the channel.

All of the above gives a reason to assume the presence of an alternative TRPM8-mediated molecular pathway for the implementation of the effects of GC hormones.

In this study we used budesonide, prednisolone, flunisolide, fluticasone propionate, hydrocortisone, dexamethasone, beclomethasone dipropionate, and triamcinolone acetonide as the most popular synthetic GCs prescribed for the treatment of chronic obstructive pulmonary disease, bronchial asthma, and allergic rhinitis in clinical practice [10–16].

Since molecular docking approaches are promising in the study of drugs, it was decided to focus on a detailed study of the characteristics of GC binding to TRPM8 [17].

The aim of this study was to conduct *in silico* screening of interactions of selected synthetic GCs with TRPM8 by the molecular docking method and to assume a possibility of forming stable complexes to determine potential ligands that act as agonists or antagonists.

MATERIALS AND METHODS

Information about the structure of ligands in sdf format was obtained from the PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/, access date: 01.10.2023).

The TRPM8 protein model was downloaded from the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/entry/Q7Z2W7, access date: 01.10.2023). Since the full-length structure of the receptor is a homotetramer with a total size of 4.5 thousand amino acid residues, for subsequent structural optimization of the protein and rapid molecular docking, only 1 subunit (PDB: AF-Q7Z2QW) in pdb format was used.

Modeling of intermolecular interactions was carried out using two different programs: AutoDock 4.2 designed to search for a local minimum energy using a genetic algorithm, and MOE 2022.02 (Molecular Operating Environment) [18], which is a complex software consisting of various modules, which allows to conduct full-fledged research in the field of computer-aided drug design of any complexity without using third-party services.

To predict potential molecular cavities and the coordinates of their centers, the PrankWeb web server (https://prankweb.cz/, access date: 01.10.2023) was used [19–21]. These coordinates were selected for the correct orientation of the Grid Box (a three-dimensional lattice within which the search and analysis of interactions between ligands and protein targets occur). Modeling intermolecular interactions with subsequent calculation of the affinity of GCs for TRPM8 was carried out by rigid docking, that is, without changing the conformations of the side chains of amino acid residues in the molecular cavity and the ligand itself. Docking took place according to the standard algorithm with generation in 100 epochs.

The first step before molecular docking is as follows: loading the target protein into the MGLtools working field, removing water molecules, and adjusting the degree of protonation (adding polar hydrogen atoms) to the protein chain at the sites of potential bonds with ligands. Next, the ligand is added in pdbqt format. The second stage is to apply the Grid

Box using the coordinates and dimensions obtained in PrankWeb. The work used a 40 x 40 x 40 Grid Box size: with an interval of 0.375 Å (default size and interval). The third stage is to search for possible conformations of the protein – ligand complex, that is, to perform the docking itself. After docking, a dlg file is created with detailed information about the formed complexes (complex location coordinates, binding energy, RMSD (root mean square deviation of atomic positions). The final stages are the analysis and interpretation of the obtained data [22].

The research software pipeline in MOE was as follows. The first stage involved importing the downloaded protein in pdb format and ligands in sdf format. For convenience, each study was conducted separately. Using the default parameters in the QuickPrep module, primary optimization of the protein was carried out, consisting of its protonation and correction of structural errors (for example, breaks). Next, partial charges were applied in the Partial charges module. The final stage of structural optimization was the implementation of protein energy minimization in the Energy minimization module, General protocol. The protocol parameters were saved by default: forcefields – inherited from the force field settings (described below), cell – no periodicity, constraints – rigid water molecules option is selected, gradient – 0.1 RMS kcal / mol / Å^2. To parameterize atoms and covalent and non-covalent interactions, the Amber14:EHT[1] force field was used, and the behavior of the solvent (water) was modeled by the Generalized Born method.

Before performing molecular docking itself, a search for the binding site was carried out using the Site Finder module with the Solvent option enabled. The experiment used the third binding site found, containing Tyr745, a critical amino acid required for channel activation. The Select Contact Atoms (selects atoms at a distance of 4.5 Å) and Select Residues in SE (selects only residues included in the binding site) options were selected and the Dummies option was executed to overlay dummy atoms, assigning the LP element to hydrophobic atoms and LPA to hydrophilic atoms (having a free pair of electrons) and also optimizing the temperature of the atom.

Molecular docking was carried out according to the General protocol. The Receptor and Solvent Atoms option was selected as the receptor, the binding site was Dummies, the ligand was the loaded ligand molecule (Ligand Atoms). The generation parameters were as follows: Placement – Triangle Matcher (Method), Affinity dG (Score), 300 Poses; Refinement – Induced fit (Method), GBVI/WSA dG (Score), 1 Pose.

A detailed description of the algorithms used for generating conformations and calculating energy before and after structural optimization is not provided in this article. As a result of docking, the most stable conformation with the lowest binding energy was extracted.

To conduct a comparative analysis between the obtained ligand conformations in AutoDock 4.2 and MOE, RMSD was calculated in the LigRMSD web service (https://ligrmsd.appsbio.utalca.cl/, access date: 01.10.2023) [23]. RMSD is a measure of the average distance between atoms (backbone, excluding H atoms) in superimposed molecules. This parameter allows to objectively assess the relative positions of ligands predicted by different methods. Based on the literature data, the threshold RMSD value was chosen to be 3Å [24].

Some of the resulting complexes were visualized using the PyMol visualization software [25] and the built-in Ligand Interactions module to construct 2D maps of interactions of ligands with amino acid residues.

The main task of using the AutoDock and MOE algorithms in our work was to assess the reproducibility of the results of molecular docking carried out by two different methods.

RESULTS

To operate the AutoDock protocol and construct the Grid Box, the coordinates of 8 putative molecular cavities were obtained with probability score values from 0.0003 (corresponding to the lowest quality of the forecast) to 0.497 (for the highest quality of the forecast). The molecular cavity with the highest probability score was selected due to the presence of the Tyr745 residue. This pocket also contains another important residue, Arg1008. According to the PrankWeb prediction, the molecular cavity is formed by amino acid residues numbered: 738, 741, 742, 745, 777, 778, 781, 782, 785, 802, 839, 842, 845, 849, 1004, 1005, 1008, 1013, 1016. The presented results

[1] This force field unites parameters of the Amber ff14SB force field for proteins and nucleic acids and parameters of the Extended Hueckel Theory for simple organic compounds in the MOE 2022.2 software package.

generally correspond to the literature data, with the exception of the absence in the pocket annotation of the Ala1009 residue, which, like Arg1008, acts as a stabilizer for the native ligand – menthol [26].

Due to the lack of a clear distinction between binding sites for agonists and antagonists, it is difficult to select one molecular cavity for the study and draw final conclusions based only on the *in silico* assessment of interactions [27]. Therefore, this study is of a screening nature, that is, it is aimed at selecting potentially suitable ligands for subsequent experiments.

The results of molecular modeling were obtained for each of the GCs (Table 1). For prednisolone, flunisolide, budesonide, beclomethasone dipropionate, and hydrocortisone, the difference in the minimum binding energy obtained by different programs was less than 1 kcal / mol, which, without taking into account chemical bonds, indicates an approximate similarity of the results obtained by the AutoDock and MOE programs, which can be explained by a similar evaluation function.

Table 1

Binding energy of complexes and RMSD of the resulting conformers, kcal / mol

Glucocorticoid	AutoDock	MOE	RMSD (Å)
Prednisolone	–7.25	–7.76	0.83
Flunisolide	–7.76	–8.26	1.62
Budesonide	–8.65	–8.58	2.54
Dexamethasone	–9.35	–7.99	4.68
Fluticasone propionate	–5.31	–7.93	6.64
Hydrocortisone	–7.65	–7.94	6.98
Triamcinolone acetonide	–11.09	–7.92	42.77
Beclomethasone dipropionate	–8.88	–8.76	43.04

Note. Ranked by RMSD, smaller values are better.

For prednisolone, flunisolide, and budesonide, the RMSD was within 3Å, indicating a relatively close relationship between the molecules, despite the use of different approaches to the generation of complexes. The similarity of conformations indicates the reproducibility of the results for GC data when analyzed by two different programs. However, information about the position of molecules is insufficient for a reliable analysis, since both ligands and amino acid residues in different programs are interpreted with different force fields and with different pH, which, in turn, is manifested by different structural interactions of ligands with amino acid residues (Fig.1).

For example, MOE showed the interaction of prednisolone with Arg842, while in AutoDock, this GC interacted with 5 residues: Leu778, Asp781, Glu782, Ile846, and Arg100. Flunisolide and budesonide interacted with almost the same amino acid residues as prednisolone, but in different combinations.

Based on these results, several conclusions can be drawn. The molecular structures of the majority of the selected GCs are very similar and differ in the presence or absence of hydroxyl groups in certain positions. Therefore, firstly, the amino acid residues for the previously mentioned ligands in the binding site are similar, and, secondly, the differences are due to the presence or absence of hydroxyl groups in a certain position, as well as different degrees of protonation. These conclusions are more typical of the results obtained in MOE, since conformational variability of both the binding site and the ligand itself is possible in this program.

The conformation of beclomethasone dipropionate with TRPM8, modeled in MOE, deserves special attention (Fig. 2). This conformation is characterized by two key features despite high RMSD relative to the complex generated in AutoDock. Firstly, interaction occurs with the key amino acid Tyr745 [27] via the H-π (hydrogen) bond. Secondly, this is the lowest binding energy calculated by this software, demonstrating the most stable binding of beclomethasone dipropionate with TRPM8. Interest in the beclomethasone dipropionate – TRPM8 complex in MOE is due to the fact that this is the only conformation where the GC forms a bond with Tyr745. Since molecular docking in AutoDock is rigid, that is, there are no conformational changes in the ligand and binding site, and MOE takes into account this "mobility" in calculations (the Induced Fit protocol was used), these results should be considered as more plausible and potentially suitable for future molecular docking with the assessment of the binding strength of the complex over time and the use of force fields. This need is due to the fact that most molecular docking algorithms take into account only partial charges of the entire ligand molecule, ignoring its individual functional groups, while most GCs have polar solvate groups (propionic, acetonide), which can play a key role in the position of the molecule.

The comparative analysis of AutoDock 4.2 and MOE findings revealed that RMSD values of other ligand positions (fluticasone propionate, hydrocortisone, triamcinolone acetonide, dexamethasone) were significantly higher. This makes it difficult to definitively interpret the resulting interactions for these GCs. So, for these ligands, it would be correct

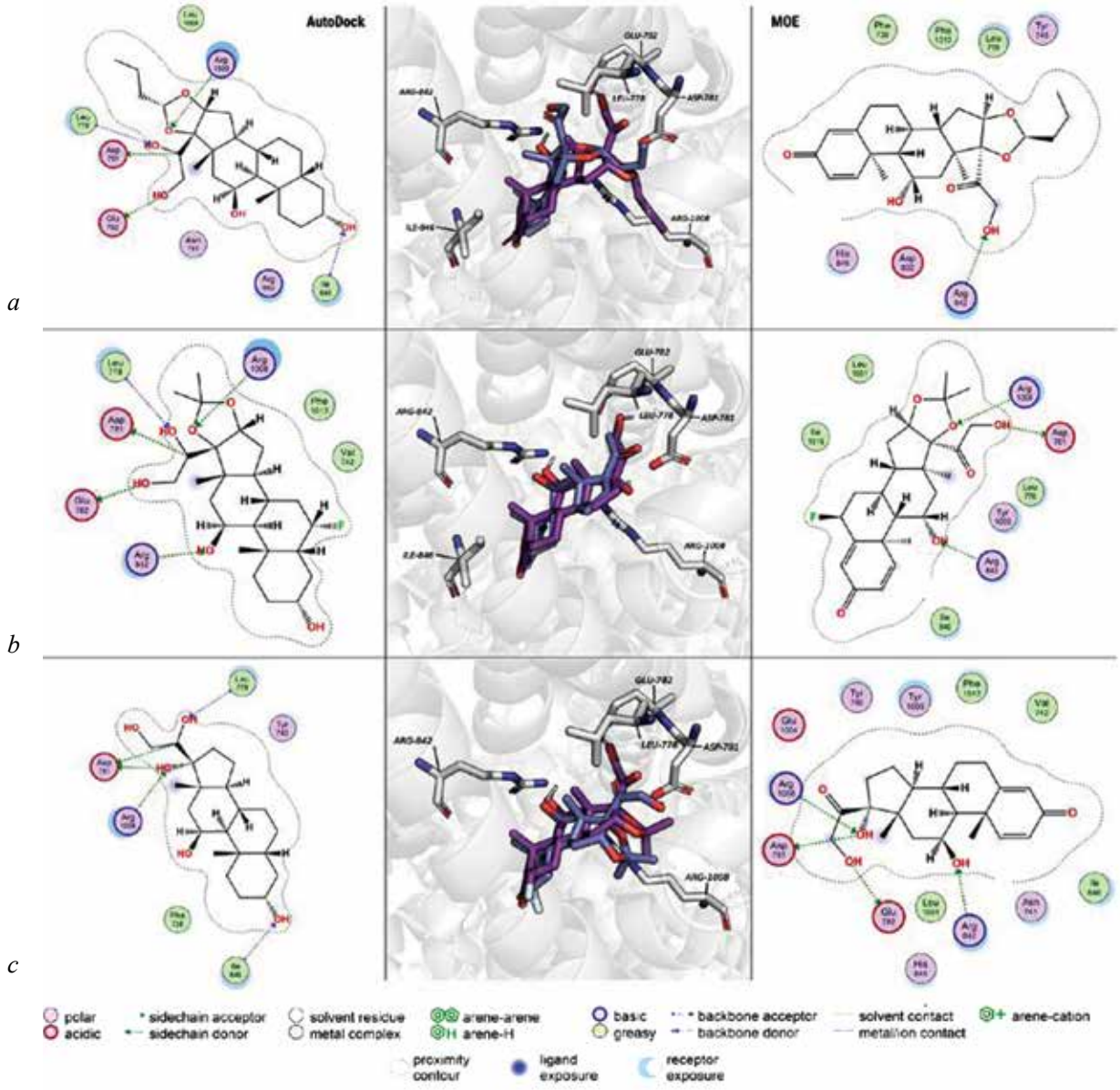

Fig. 1. Position of ligands in the TRPM8 binding site and a 2D map of structural interactions of ligands with site residues: *a* – budesonide, *b* – prednisolone, *c* – flunisolide; on the left – AutoDock, on the right – MOE; blue color marks the positions of molecules obtained in AutoDock 4.2, purple color marks the positions of molecules obtained in MOE

to carry out an additional analysis using *ab initio* methods. The visual analysis of all conformations obtained in AutoDock allow to conclude that the rigid orientation of the GC molecules took place mainly along the steroid ring with minimal deviations relative to each other. As for the conformations modeled in MOE, the differences in them are more significant, which is due to the inclusion of minor differences (functional groups, conformational isomerization of the ligand) in the ligand structures.

The absolute energy values calculated for various conformations, on the one hand, are far from actual values; however, on the other hand, they allow to consider them from a relative point of view and compare the binding energies of different molecules, ranking their degree of affinity relative to each other. Therefore, the series of ligands according to the degree of affinity for TRPM8 (from the greatest to the lowest, from the lowest energy level to the highest) following the results of scoring in AutoDock is as follows: triamcinolone

acetonide, dexamethasone, beclomethasone dipropionate, budesonide, flunisolide, hydrocortisone, prednisolone, and fluticasone propionate. According to the results of scoring in MOE, the ligand series is the following: beclomethasone dipropionate, budesonide, flunisolide, dexamethasone, hydrocortisone, fluticasone propionate, triamcinolone acetonide, and prednisolone. This series shows that beclomethasone, budesonide, flunisolide, hydrocortisone, and fluticasone propionate / prednisolone appear in the same order, which represents a very good correlation of the results (6 out of 8). Based on the series, we are planning to study the effects of synthetic GCs on TRPM8 *in vitro*.

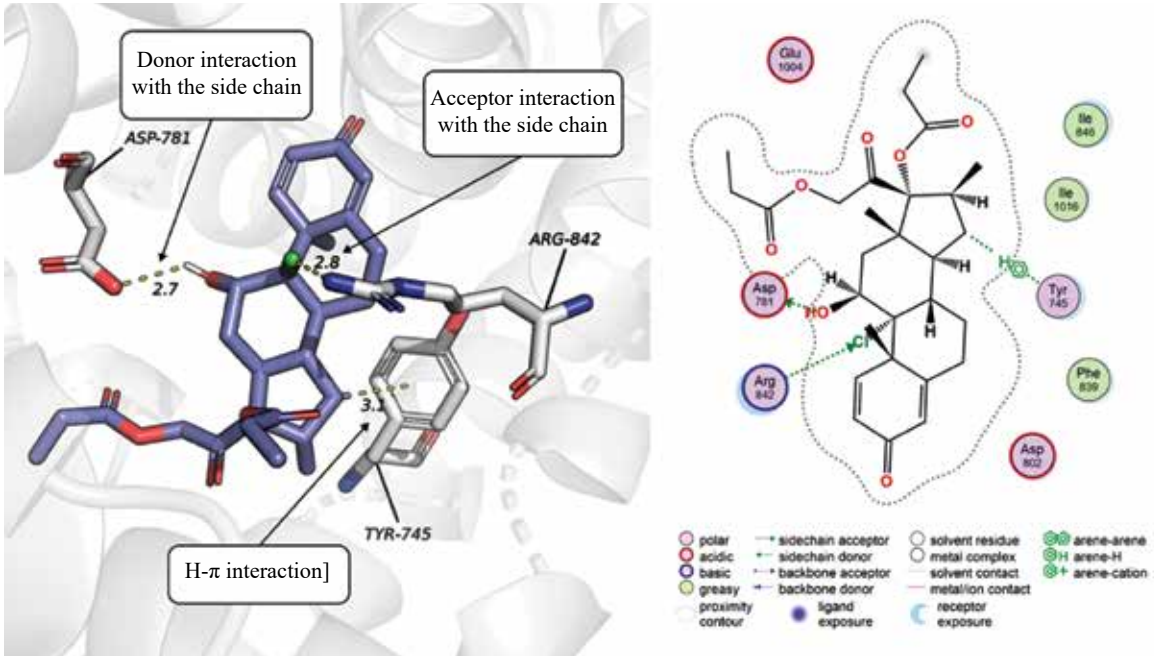

Fig. 2. 3D visualization of the beclomethasone dipropionate – TRPM8 complex, obtained in MOE, with a 2D graph of interactions: the arrows on the left and the yellow dotted line duplicate the interactions shown in the 2D graph; bond length units are given in Angstroms (Å)

DISCUSSION

The conducted study demonstrates general patterns in the molecular interaction of various synthetic GCs with the target, obtained by two different methods. For prednisolone, flunisolide, and budesonide, the RMSD value was less than 2.5Å (±0.1Å), indicating conformational similarities and reproducibility of the results in both AutoDock and MOE. It is worth noting that in the molecular cavity, various amino acid residues served as binding sites, with the exception of Tyr745, which may characterize the antagonistic potential of prednisolone, flunisolide, and budesonide. For GCs, whose conformations differed significantly, the formation of hydrogen bonds with the amino acid residue Tyr745 was also ignored, which is consistent with other results.

An exception to the list of GCs was beclomethasone dipropionate, which ultimately formed a hydrogen bond with Tyr745. The study made it possible to select the most promising GCs suitable for further analysis using molecular dynamics methods, which will make it possible to clarify the stability of GC complexes with TRPM8. A final confirmation of the ability of GCs to not only form complexes with the TRPM8 receptor, but also to inhibit it should be obtained through *in vitro* experiments.

CONCLUSION

Detailed mechanisms of the anti-inflammatory effect of GCs mediated through the TRPM8 ion channel remain a big question for our research group. However, if experimentally confirmed, the possibility of pharmacological modulation of TRPM8 by GCs will allow to optimize approaches to personalized use of GCs and take a different look at the therapeutic potential of these hormones, including their effect in the treatment of chronic obstructive pulmonary disease, respiratory diseases, and cold-induced respiratory diseases.

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Received 16.01.2024;
approved after peer review 18.04.2024;
accepted 14.05.2024