**https://doi.org/10.18524/2077–1746.2023.2(53).293324**
**УДК 547.892/577.322.21;615(213/212.3)**

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## MOLECULAR INSIGHTS INTO PROPOXAZEPAM INTERACTION WITH TRPV1 RECEPTORS: A DOCKING ANALYSIS

In this study, we used molecular docking modelling to investigate the interaction between propoxazepam (new analgesic), and the transient receptor potential vanilloid type 1 receptors. Propoxazepam has the necessary pharmacophoric features of the pharmacophore model of TRPV1 ligands. Propoxazepam creates hydrogen bond between TYR511 of the TRPV1 receptor as well as referent ligand SB-366791.

**Key words:** docking; propoxazepam; TRPV1 receptor; pain; binding; 1,4-benzodiazepines

Pain is a frequent reason for patients to go to a medical facility [18]. An alternative way to treat pain without addiction is by targeting receptors that are at the origin of the pain pathway, such as transient receptor potential (TRP) ion channels. TRPV1, the vanilloid TRP channel subfamily's founding member, is one of the most popular targets for pain therapy. The need for TRPV1 inhibitors that are specific for pain treatment goes beyond pain treatment and covers other diseases related to this channel, such as psychiatric disorders [15]. TRPV1(Transient receptor potential vanilloid 1) is a member of the vanilloid TRP (transient receptor potential) channel family, similar to voltage-gated potassium channels, and it is present in both the central nervous system and peripheral blood vessels. Inflammatory bowel syndrome (IBS), osteoarthritis, rheumatoid arthritis, postherpetic neuralgia (PHN), and cystitis are all

pathophysiological conditions that TRPV1 is involved in. This makes understanding this receptor essential for the treatment of many different disorders. Reducing pain perception and itch sensation in pathological conditions can be achieved through the use of TRPV1, which has been shown to be a promising therapeutic target. There are two ways that TRPV1 can be targeted pharmacologically, either it can be desensitized by vanilloids by agonists or blocked by antagonists. TRPV1 agonists have therapeutic effects by desensitizing pain-conducting nerve fibres, which is a contributing factor to analgesic effects. Desensitization to TRPV1 agonists (eg, capsaicin and resiniferatoxin) is a powerful approach to alleviate nociceptive behavioural symptoms in animal models of chronic pain [4,8,17]. The vanillyl group is the predominant ingredient in most potent TRPV1 agonists reported until now, but several examples demonstrate that this group can be substituted by similar chemical groups. Recently, it was discovered that the TRPV1 agonist MDR-652 has a 3-fluoro-4-(hydroxymethyl)phenyl group instead of a vanillyl group (table 1). TRPV1 antagonists exhibit analgesic and anti-inflammatory effects in neuropathic pain [4]. Heat, protons, and chemical ligands are the three modes of TRPV1 activation that TRPV1 antagonists selectively block [7].

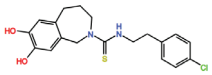
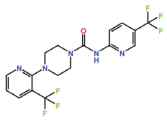
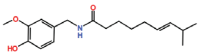
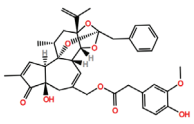
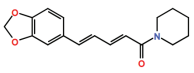
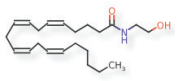
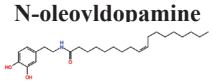
In vitro and in vivo studies revealed that SB-366791 (N-(3-methoxyphenyl)-4-chlorocinnamide), the cinnamamide antagonist, had a high affinity for inhibiting TRPV1, but it did not exhibit little effect on a panel of 47 different targets, including G-proteins and voltage-dependent calcium channels. SB-366791, a TRPV1-specific inhibitor, is a promising candidate for treating TRPV1-related pain. The use of SB-366791 has been implicated in treating TRPV1-mediated inflammatory pain by blocking glutamatergic transmission in a specific set of neurons through a pre-synaptic TRPV1-dependent mechanism after peripheral inflammation [15]. The innovative drug «Propoxazepam», which was created by the scientists of the O.V. Bogatsky Institute of Physics and Chemistry of the National Academy of Sciences of Ukraine and CAR «INTERCHIM», has an original pharmacodynamic profile, since it simultaneously inhibits acute and chronic pain and exhibits anti-inflammatory and anticonvulsant effects [2]. Propoxazepam also interacts with other biological targets responsible for the course of pain: glycine receptors, dopaminergic system, NMDA receptors, alpha-1 adrenoceptors [1]. **The aim of the study:** the study of interactions of ligands with the TRPV1 receptor by molecular docking and analysis of components of these interactions.

## Materials and methods

In order to study the binding energy of the TRPV1 receptor with the researched compounds 8GFA – Cryo-EM structure of human TRPV1 in complex with the analgesic drug SB-366791 was utilized. The protein was modelled by using Protein Preparation of Schrödinger Suite; to prepare the protein structure, hydrogen atoms were added and hydrogen bonds were optimized. The ligands were prepared by

Table 1

**General information about agonists and antagonists of TRPV1 receptor (Schemes of chemical structures have been created by Draw Structure NIH)**

| | Ligands of TRPV1 receptor | Description |
|---|---|---|
| Antagonist | **Capsazepine**  *Draw Structure NIH* | Capsazepine consists of a 2,3,4,5-Tetrahydro-1H-2-benzazepine-7,8-diol in place of the vanillyl group. Blocking the activation of TRPV1 and transient receptor potential cation channel subfamily A member 1 (TRPA1) is possible with it [3]. |
| | **JNJ – 17203212**  [9] | It is an TRPV1 receptor antagonist. The drug exerts potent antinociceptive and antitussive actions. It is developing by Johnson & Johnson for the treatment of pain and cough [9]. |
| Agonist | **Capsaicin**  [10] | Capsaicin, a botanical irritant naturally present in chili peppers, is synthetically produced for pharmaceutical uses. TRPVI, a complex of ion channels and receptors that is expressed on nociceptive nerve fibres in the skin, is agonised by this substance [10]. Capsaicin's initial exposure activates TRPV1 ligand gated channels on the primary afferent nociceptive neurons, it leads to depolarization, the beginning of an action potential, and the transfer of pain signals to the spinal cord. Following this, there is a prolonged refractory period called desensitization. If the dose is too much, it leads to a more durable local desensitization called defunctionalization [8]. |
| | **Resiniferatoxin (RTX)**  [11] | *Euphorbia resiniferous* (cactus-like plant) is the source of Resiniferatoxin (RTX or RTX-107) which is an anti-inflammatory vanilloid. This substance acts as an agonist for the transient receptor potential vanilloid 1 (TRPV1). Desensitizing the TRPV1 receptor causes resiniferatoxin to cause analgesia [11]. |
| | **Piperine**  [12] | The seeds of black pepper (*Piper nigrum*) contain a simple and pungent alkaloid known as piperine. Piperine activates TRPV1 by directly interacting with the pore-forming S6 segment, in a manner distinct from that of capsaicin [6]. The properties of piperine include immunomodulation, anti-ulcer, anti-oxidation, anti-carcinogenic, anti-asthmatic, anti-inflammatory and anti-amoebic [12]. |
| | **Anandamide**  [13] | Anandamide is a neurotransmitter that is involved in the regulation of cannabinoid receptors. It has an anti-proliferative effect that is associated with a decrease in cells in the S phase of the cell cycle. In mice, Anandamide has a positive impact on motivation and eating behaviour [13]. |
| | **N-oleoyldopamine**  [14] | N-oleoyldopamine is a lipid compound that comes from the mammalian brain. It has been proven that it is a capsaicin receptor (TRPV1) agonist. The capsaicin receptor activation leads to glutamate release and postsynaptic firing in the paraventricular nucleus. Calcium influx can be induced by N-oleoyldopamine in TRPV1-transfected HEK293 human embryonic kidney cells, as reported [14]. |

LigPrep module of Schrödinger suite before proceeding for docking [16]. The docking and QSAR prediction were carried out with propoxazepam, its possible metabolite 3-hydroxopropoxazepam, diazepam, oxazepam, SB-366791, RTX, capsazepin, capsaicin. The QSAR build model using the automated QSAR panel of Maestro Schrödinger Suite. For building QSAR model we used 408 TRPV1 antagonists being comparable between each other. List of these substances we took from Supporting Information of the article of Pharmacoinformatics Research Group Univ.-Prof. Dr. Gerhard F. Ecker. Department of Pharmaceutical Sciences Division of Pharmaceutical Chemistry University of Vienna. The dataset had an IC50 range between 0.4 and 17490 nM. The capsazepine activity is determined to be 100 nM in this assay type. This value was used as a threshold to categorize compounds into active and inactive in their ability to block the receptor after capsaicin activation. A dataset with 201 active compounds and 207 inactive compounds was produced as a result [18].

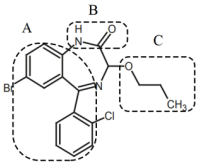
*Molecular docking:* The location of the referent ligand (the analgesic drug SB-366791) was used to determine automatic binding site detection. The quality of geometric contacts and their energy were used to calculate the interaction between protein and ligand complexes. The following formulas were used to rank the ligands based on their G-scores G-score = (0.05*vdW) + (0.15*Coul)+ Lipo + Hbond + Metal + Rewards + RotB + Site (1), where vdW was the Van der Waals energy, Coul represents the Coulomb energy, Lipo term explains the Lipophilic, Rewards describes the favorable hydrophobic interactions, Hbond means Hydrogen-bonding term, Metal gives the information about metal-binding RotB tells about penalty for freezing rotatable bonds and Site defines polar interactions in the active site. To perform binding free energy calculations for MM-GBSA, the Prime module in the Schrödinger Suite was utilized. The binding energy is calculated according to the equation: DG bind = E_complex(minimized) – E_ligand(minimized) - E_receptor(minimized) [16].

## Results and discussion

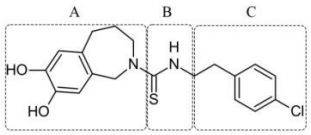
**Pharmacophore modelling.** Capsaicin-like TRPV1 ligands have a well-defined pharmacophore consisting of a sequential arrangement of three chemical elements: aryl interaction head, neck (H-bond interaction linker), and tail group (the lipophilic chain). It is possible to identify these pharmacophoric features in TRPV1 agonists and antagonists. The vanilloid group (4-hydroxy-3-methoxyphenyl) is located in the head region of capsaicin ligand [4]. A wide variety of synthesized TRPV1 ligands such as catechol-containing structures, 2,3-dihydro-1,4-benzodioxine derivatives, 3-fluoro-4-(methylsulfonylamino) phenyl-containing structures, and chalcones have been reported in recent decades that contain other groups rather than a vanilloid moiety [5].

We built a pharmacophore model based on a list of ligands taken from the Supporting Information of the article of Research Group Dr. Gerhard F. Ecker. In the obtained pharmacophore model, it is possible to distinguish such parts of the ligand as aryl interaction region A, amide B-region, and the hydrophobic chain C.

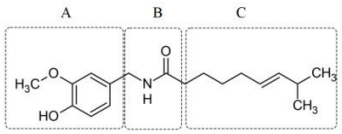
The propoxazepam we are studying is one of the alkoxy derivatives of 1,4-benzodiazepine. Propoxazepam has the necessary pharmacophoric features: region A-benzene ring, which is connected to a seven-membered diazepine ring and aryl substituent in position 5; region B – the amide group (NH-C=O). This group contains a hydrogen atom that can act as a hydrogen bond donor and the carbonyl oxygen that can act as a hydrogen bond acceptor. This makes it an important site for interactions with other molecules, such as receptors or other proteins in the body; region C – alkoxy group (fig. 1).



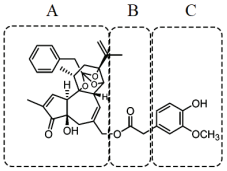

**Propoxazepam** (subject of investigation)




**Capsazepin** (TRPV1 antagonist)




**Capsaicin** (TRPV1 agonist)




**Resiniferatoxin (RTX)** (TRPV1 agonist)

*Fig. 1 Structures of ligands pharmacophoric features prepared by Maestro Schrödinger Suite. (**A** - Aryl interaction head, **B** – H -bond interaction linker, **C** – Hydrophobic tail)*

**Docking analyses using GLIDE module.** The study of the docking of ligands with the active centres of the protein was carried out using the advanced molecular docking program Maestro Schrödinger Suite to determine the binding affinity of the compounds. Docking results allowed for the determination of the interaction's gscore values, as well as its components – hydrophobic interactions and hydrogen bonding for researched ligands in the binding site of TRPV1 receptor were determined (fig. 2). Molecular docking was provided for each molecule of ligand per subunit of hTRPV1 tetramer.The interactions within each binding site are determined by the contribution of specific amino acid residues to the overall process, which determine the strength and type of the interaction. The docking results were analysed by using docking score (calculated noncovalent three-dimensional interactions between a ligand and a protein).

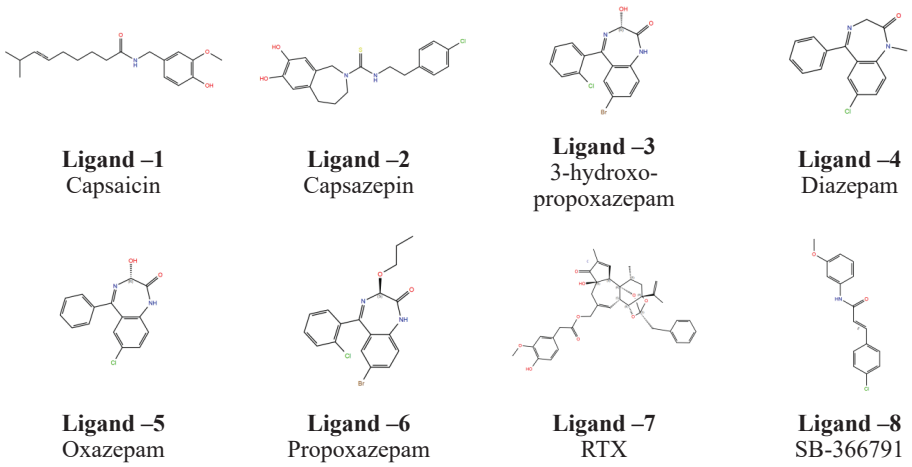



*Fig. 2 Structures of the investigated compounds prepared by LigPrep module*

Table 2

**Docking scores using GLIDE module chain A of TRPV1 receptor**

| Ligand | docking score | gscore | lipo[1] | hbond[2] | evdw[3] | ecoul[4] | other interaction types[5] |
|---|---|---|---|---|---|---|---|
| 1 | -7.71 | -7.71 | -3.73 | -0.30 | -39.98 | -9.01 | -0.33 |
| 2 | -6.39 | -6.40 | -3.18 | 0 | -32.18 | -4.09 | -0.99 |
| 3 | -6.49 | -6.49 | -2.43 | 0 | -29.38 | -6.11 | -1.68 |
| 4 | -7.66 | -7.66 | -3.05 | -0.21 | -28.39 | -4.67 | -2.29 |
| 5 | -6.82 | -6.82 | -2.60 | 0 | -26.09 | -5.17 | -2.41 |
| 6 | -7.30 | -7.30 | -3.15 | -0.57 | -35.73 | -4.52 | -1.12 |
| 7 | -8.23 | -8.23 | -4.02 | -0.32 | -46.03 | -4.67 | -0.9 |
| 8 | -9.54 | -9.54 | -4.48 | -0.32 | -42.82 | -6.50 | -1.64 |

[1]**lipo** (lipophilic contact), [2]**hbond** (hydrogen bond), [3]**evdw** (Van der Waals energy), [4]**ecoul** (Coulomb energy), [5]**other interaction type** (metal-binding + rewards + penalty for freezing rotatable bonds + polar interactions in the active site)

The findings indicate that the reference compound SB-366791 has the lowest docking scores and MMGBSA free energy of binding across binding sites on all four chains, which means that this compound has the best affinity for the TRPV1 receptor than the other ligands. Specifically, the docking score of propoxazepam (–7.30 kcal/mol) indicates a stronger interaction with the TRPV1 receptor compared to oxazepam (–6.82 kcal/mol), 3-hydroxopropoxazepam (–6.49 kcal/mol), and

capsazepin (–6.39 kcal/mol). The docking score of propoxazepam in chain C and B is lower than that of diazepam, resulting in stronger interaction than that of diazepam (table 2). The binding energy were analysed by using MMGBSA_dG_ Bind (Molecular Mechanics Generalized Born Surface Area) (table3).

Table 3

**Energy of binding of receptor ligand complex calculated using Prime MMGBSA method chain A of TRPV1 receptor**

| Li-gand | MMGBSA_ dG_Bind | Coulomb[1] | Covalent[2] | H-bond[3] | Lipo[4] | Packing[5] | Solv_ GB[6] | vdW[7] |
|---|---|---|---|---|---|---|---|---|
| 1 | -61.98 | -26.45 | 2.09 | -1.53 | -29.45 | -0.44 | 37.67 | -43.88 |
| 2 | -48.45 | -15.09 | -0.46 | -0.83 | -25.12 | -2.39 | 27.59 | -32.15 |
| 3 | -38.01 | -18.48 | 7.67 | -1.01 | -15.89 | -0.63 | 20.97 | -30.65 |
| 4 | -43.14 | -13.40 | 4.54 | -0.42 | -21.35 | -0.72 | 16.18 | -27.95 |
| 5 | -37.51 | -16.81 | 4.18 | -1.02 | -18.44 | -0.52 | 23.46 | -28.37 |
| 6 | -40.96 | -15.60 | 8.51 | -1.27 | -23.59 | -0.59 | 30.53 | -38.95 |
| 7 | -61.12 | -13.09 | 4.96 | -0.70 | -36.81 | -0.25 | 41.22 | -56.45 |
| 8 | -71.63 | -18.84 | 0.799 | -0.77 | -29.01 | -1.22 | 24.22 | -46.83 |

[1]**Coulomb** (Coulomb energy), [2]**Covalent** (Covalent binding energy), [3]**H-bond** (Hydrogen-bonding correction), [4]**Lipo** (Lipophilic energy), [5]**Packing** (Pi-pi packing correction), [6]**Solv_GB** (Generalized Born electrostatic solvation energy), [7]**vdW** (Van der Waals energy)

Furthermore, propoxazepam demonstrates a lower value of MMGBSA free energy of binding compared to oxazepam and 3-hydroxopropoxazepam. When considering the increase in the free energy of interactions, the ligands can be ranked as follows: SB-366791 > Capsaicin > RTX > Capsazepin > Propoxazepam > Diazepam > 3-hydroxopropoxazepam > Oxazepam. However, in chains B, C, and D, propoxazepam has a better MMGBSA free energy value than capsazepin (table 3). It's important to note that, as per the glide module, propoxazepam exhibits the largest contribution of hydrogen bonds in the energy of interaction with the receptor. Additionally, the prime MMGBSA method also shows that one of the highest contribution hydrogen bonds in free energy of binding belongs to propoxazepam, second only to capsaicin.

Propoxazepam establishes two hydrogen bonds: one involving the NH group of the amide (resulting in a hydrogen bond interaction with the linker-neck) and THR550 of the protein, and another between oxygen of the alkoxy group (hydrophobic tail) and TYR511 of the TRPV1 receptor. Other benzodiazepines also form hydrogen bonds with the receptor. For instance, 3-hydroxopropoxazepam and oxazepam create a hydrogen bond between the hydroxyl group of the diazepam ring and TYR511, and 3-hydroxopropoxazepam additionally establishes another hydrogen bond between the NH group of the amide group of the diazepine ring and THR550. Diazepam

interacts with the receptor through hydrogen bonding by utilizing oxygen of the amide group with TYR511. Ligands with confirmed effects on the TRPV1 receptor also engage in interactions with the protein by forming hydrogen bonds with the same amino acids as the benzodiazepines, namely THR550 and TYR511. Capsaicin uses oxygen of the amide group to form a hydrogen bond with THR511 of the receptor and the hydroxyl group of the benzene ring with GLU570. Capsazepin establishes a hydrogen bond via its OH group with THR550. In the interaction between RTX and the TRPV1 receptor, TYR511 plays a crucial role as this amino acid forms a hydrogen bond with the ester group of region B. Regarding the reference compound SB-366791, it forms a single hydrogen bond between the oxygen of its amide group and TYR511 (fig. 3).

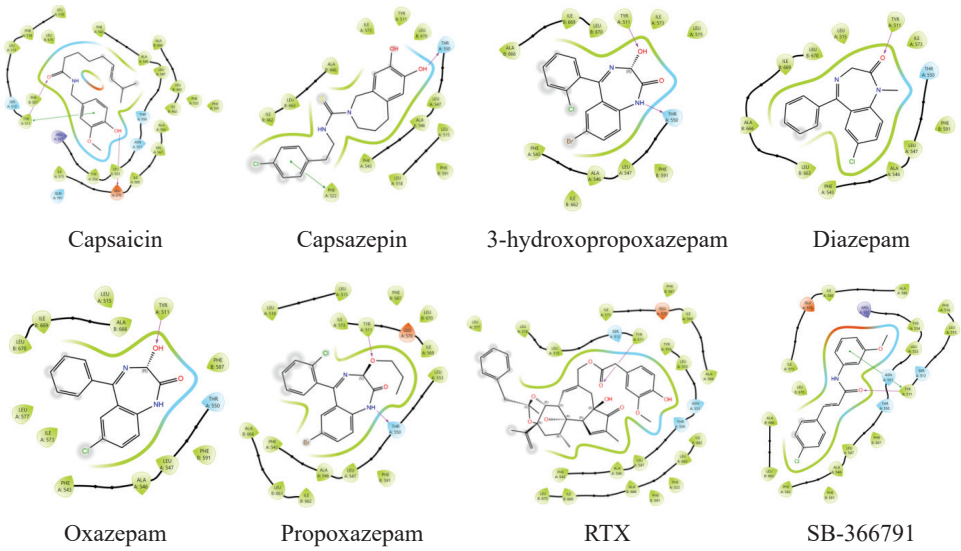



*Fig. 3 Visualization of location in specific binding site of investigated ligands in the binding site of TRPV1 prepared by Maestro Schrödinger Suite*

**QSAR analyse.** In the research, the model kpls_desc_19 was chosen because it demonstrates the highest score 0,63. This model has an R-squared value for the regression (the coefficient of determination) is 0.6445, which is the second-highest among the models. R-squared measures how well the model fits the training data, and a value of 0.6445 indicates a reasonably good fit. «kpls_desc_19» has an RMSE of 0.6601, which is not the lowest but is still competitive. RMSE measures the average prediction error, and lower values are preferred. While it's not the lowest RMSE, it's still within an acceptable range. «kpls_desc_19» has a Q^2 value of 0.6430, indicating good predictive performance on new, unseen data. This suggests that the model is likely to make accurate predictions beyond the training dataset. Q^2 MW

(Null hypothesis): Chosen model has a Q^2 MW value of 0.0042, which is positive and suggests that it performs significantly better than a null hypothesis model. This is important because it indicates that the model has genuine predictive power (fig.4).

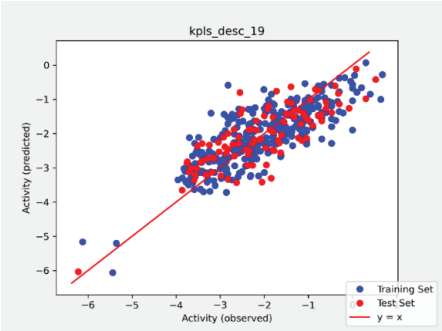



*Fig.4 The best model kpls_desc_19 created by Maestro Schrödinger Suite was used for observation and prediction of training and test set activity.*

Because of the minus sign, higher pIC50 values indicate exponentially more potent inhibitors. Diazepam and Oxazepam have the lowest predicted pIC50 values, indicating that the model predicts them to be less potent among the compounds. Propoxazepam has the highest predicted pIC50, it means that the model predicts it to be the most potent among the compounds (table 4).

Table 4

**Predicted IC50 of investigated compounds using best model kpls_desc_19**

| | 3-hydroxo-propoxazepam | Diazepam | Oxazepam | Propoxazepam |
|---|---|---|---|---|
| Pred *pIC50* | -1.958 | -2.283 | -2.253 | -1.115 |

Lower pIC50 values (closer to negative infinity) suggest lower potency, meaning that the compound has a weaker affinity for the target and is less likely to affect the target's activity significantly. In our case, with a pIC50 value of –1.115, propoxazepam is predicted to have relatively low potency or affinity for TRPV1, but this value is higher than other ligands.

**Conclusions:**

1. Propoxazepam has the necessary pharmacophoric features of pharmacophore model of TRPV1 ligands: aryl interaction head (benzene rings), H -bond interaction linker (the amide group (NH-C=O)), hydrophobic tail (alkoxy group).
2. The docking score of propoxazepam (–7.30 kcal/mol) indicates a stronger interaction with the TRPV1 receptor compared to oxazepam (–6.82 kcal/mol), 3-hydroxopropoxazepam (–6.49 kcal/mol), and capsazepin (–6.39 kcal/mol).

Propoxazepam creates hydrogen bond with TYR511 of the TRPV1 receptor as referent ligand SB-366791. Propoxazepam exhibits one of the largest contributions of hydrogen bonds in the energy of interaction with the receptor.

3. According to QSAR modelling, all studied compounds (3-hydroxo-propoxazepam, diazepam, oxazepam, propoxazepam) have low pIC50 values, which could indicate a relatively low potency or affinity for TRPV1.

The research paper was received October 31, 2023

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# МОЛЕКУЛЯРНЕ УЯВЛЕННЯ ПРО ВЗАЄМОДІЮ ПРОПОКСАЗЕПАМУ З РЕЦЕПТОРАМИ TRPV1: ДОКІНГ-АНАЛІЗ

**Резюме**

**Проблема.** Знеболююча терапія залишалася концептуально незмінною після опіоїдної кризи, яка підкреслила небезпеку лікування болю опіоїдами. Рецептори TRPV1 та їх ліганди стали багатообіцяючими мішенями для лікування різних больових станів, включаючи запальний і нейропатичний біль. Подальші дослідження механізмів активації та десенсибілізації рецепторів TRPV1, а також дослідження нових лігандів з підвищеною вибірковістю та ефективністю мають потенціал для полегшення тягаря хронічного болю.

**Мета.** Вивчення взаємодії лігандів з рецептором TRPV1 шляхом молекулярного докінгу та аналіз компонентів цих взаємодій.

**Методика.** З метою вивчення енергії зв'язку рецептора TRPV1 з досліджуваними сполуками 8GFA – була використана структура Cryo-EM людини TRPV1 у комплексі з анальгетиком SB-366791. Докінг і QSAR-прогноз проводили з пропоксазепамом, його можливим метаболітом 3-гідроксопропоксазепамом, діазепамом, оксазепамом, SB-366791, RTX, капсазепіном, капсаїцином. Докінг аналіз та створення QSAR моделі проводили за допомогою Maestro Schrödinger Suite.

**Основні результати.** Пропоксазепам має необхідні фармакофорні властивості для зв'язування з рецептором TRPV1: область А-бензольне кільце; область В – амідна група (NH-C=O); область С – алкоксигрупа. Докінг-оцінка пропоксазепаму (–7,30 ккал/моль) вказує на сильнішу взаємодію з рецептором TRPV1 порівняно з оксазепамом (–6,82 ккал/моль), 3-гідроксопропоксазепамом (–6,49 ккал/моль) і капсазепіном (–6,39 ккал/моль). Розглядаючи збільшення вільної енергії взаємодії, ліганди можна ранжувати наступним чином: SB-366791 > Капсаїцин > RTX > Капсазепін > Пропоксазепам > Діазепам > 3-гідроксопропоксазепам > Оксазепам. Пропоксазепам створює два водневі зв'язки: один за участю NH-групи аміду (що призводить до взаємодії водневого зв'язку з лінкерною шийкою) і THR550 білка, а інший – між киснем алкоксигрупи (гідрофобний хвіст) і TYR511 рецептору TRPV1. Ліганди з підтвердженою дією на рецептор TRPV1 також вступають у взаємодію з білком, утворюючи водневі зв'язки з тими ж амінокислотами, що й бензодіазепіни, а саме THR550 і TYR511. Передбачається, що пропоксазепам має відносно низьку

ефективність або спорідненість з TRPV1 (прогнозований показник pIC50 становить –1,115), але це значення вище, ніж у інших лігандів.
**Висновки.** Оскільки пропоксазепам має необхідні фармакофорні властивості фармакофорної моделі лігандів TRPV1, він теоретично може зв'язуватися з цим рецептором. Пропоксазепам створює водневий зв'язок із TYR511 рецептора TRPV1 як референтний ліганд SB-366791. Пропоксазепам демонструє один з найбільших внесків водневих зв'язків в енергію взаємодії з рецептором. Відповідно до моделювання QSAR, усі досліджувані сполуки (3-гідроксопропоксазепам, діазепам, оксазепам, пропоксазепам) мають низькі значення pIC50, що може вказувати на відносно низьку ефективність або спорідненість з TRPV1. Але пропоксазепам має найвищий прогнозований pIC50, це означає, що модель передбачає, що він є найпотужнішим серед сполук.

**Ключові слова:** докінг; пропоксазепам; рецептор TRPV1; біль; зв'язування; 1,4-бензодіазепіни

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## MOLECULAR INSIGHTS INTO PROPOXAZEPAM INTERACTION WITH TRPV1 RECEPTORS: A DOCKING ANALYSIS

**Summary**
**Problem.** Pain therapy has remained conceptually stagnant since the opioid crisis, which highlighted the dangers of treating pain with opioids. TRPV1 receptors and their ligands have emerged as promising targets for the management of various pain conditions, including inflammatory and neuropathic pain. Further investigation into the activation and desensitization mechanisms of TRPV1 receptors, as well as the exploration of novel ligands with enhanced selectivity and efficacy, holds the potential to alleviate the burden of chronic pain.
**Aim.** The study of interactions of ligands with the TRPV1 receptor by molecular docking and analysis of components of these interactions.
**Methods.** In order to study the binding energy of the TRPV1 receptor with the researched compounds 8GFA – Cryo-EM structure of human TRPV1 in complex with the analgesic drug SB-366791 was utilized. The docking and QSAR prediction were carried out with propoxazepam, its possible metabolite 3-hydroxopropoxazepam,

diazepam, oxazepam, SB-366791, RTX, capsazepin, capsaicin. The docking and QSAR build model using the Maestro Schrödinger Suite.

**The main results.** Propoxazepam has the necessary pharmacophoric features to bind with TRPV1 recepor: region A-benzene ring; region B – the amide group (NH-C=O); region C – alkoxy group. The docking score of propoxazepam (–7.30 kcal/mol) indicates a stronger interaction with the TRPV1 receptor compared to oxazepam (–6.82 kcal/mol), 3-hydroxopropoxazepam (–6.49 kcal/mol), and capsazepin (–6.39 kcal/mol). When considering the increase in the free energy of interactions, the ligands can be ranked as follows: SB-366791 > Capsaicin > RTX > Capsazepin > Propoxazepam > Diazepam > 3-hydroxopropoxazepam > Oxazepam. Propoxazepam establishes two hydrogen bonds: one involving the NH group of the amide (resulting in a hydrogen bond interaction with the linker-neck) and THR550 of the protein, and another between oxygen of the alkoxy group (hydrophobic tail) and TYR511 of the TRPV1 receptor. Ligands with confirmed effects on the TRPV1 receptor also engage in interactions with the protein by forming hydrogen bonds with the same amino acids as the benzodiazepines, namely THR550 and TYR511. Propoxazepam is predicted to have relatively low potency or affinity for TRPV1 (predicted pIC50 value of –1.115), but this value is higher than other ligands.

**Conclusions.** As propoxazepam has the necessary pharmacophoric features of the pharmacophore model of TRPV1 ligands, it could theoretically bind with this receptor. Propoxazepam creates hydrogen bond with TYR511 of the TRPV1 receptor as referent ligand SB-366791. Propoxazepam exhibits one of the largest contributions of hydrogen bonds in the energy of interaction with the receptor. According to QSAR modelling, all studied compounds (3-hydroxopropoxazepam, diazepam, oxazepam, propoxazepam) have low pIC50 values, which could indicate a relatively low potency or affinity for TRPV1. But propoxazepam has the highest predicted pIC50, it means that the model predicts it to be the most potent among the compounds.

**Key words:** docking; propoxazepam; TRPV1 receptor; pain; binding; 1,4-benzodiazepines

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